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(54) Title: SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

(57) Abstract

Compounds having utility as light absorbing compounds, especially in the area of photodynamic therapy. Such compounds have formula (I), where z is = 0 or NR₁₄; R₁₄ is alkyl or substituted alkyl, R₁ is an amino acid group, aryl, or a carbonyl containing group, provided that: R4 may be taken together with R5 to form -0; R6 may be taken together with R7 to form -0; R8 may be taken together with R9 to form a polyamine group, a polyether group or OR13 where R13 is alkyl; R4 through R11 are .H, -OH, alkyl, alkylene, -OR16 where R16 is H, alkyl or -O; R to may be taken together with R11 to form -O; and R4 and R7 may together form a chemical bond and R₈ and R₁₁ may together form a chemical bond; and R₁₂ is hydrogen or lower alkyl; provided that if one z is 0, the other z is

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R SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

Background of the Invention

mploying analogs cancer porphyrin related compounds and more particularly certain invention relates to treatment and diagnosis through the use of IR imaging and photodynamic therapy chlorins and bacteriochlorins. This

concentrations and these The and/or synthesizing DNA or undergoing cell growth or nutrient uptake. retention time is not dependent on whether or not the cells fluoresce when activated by light of a specific wavelength. tissues. chemicals which kill cells retain surrounding normal tissues substances in higher premalignant longer durations than Photosensitizers are photochemically active some and malignant

injections of a photodynamic drug that accumulates in optic probes have In this new therapy, patients are given cancer being energy (PDT). cells. been combined in a procedure known as photodynamic therapy PDT has emerged as one of the most promising strategies in . 1. cancer cells in much higher concentrations than in normal and high PDT surgery sensitive drugs, lasers and fiber detection). chemotherapy, photodynamic (photosensitizing) cancer increasingly used where irradiation have failed. (including intravenous treatment Light The

activ

then

drug is

-1

an important form of cancer therapy that has chemotherapy or high energy often anesthesia and does not necessitate hospital can be performed any number contraindicated with selective treatment therapy, which cancer ü dye ţ or malignant tissues due to preferential retention of established that a laser beam directed old too it is not superficial malignancy, PDT may be curative. photodynamic allows People who are surgery, additional advantages, e.g. it cells and it has already been single patient, cancer therapies and it þ tolerate the stress of major cells by radiation may be helped optics. just local PDT is kill the cancer Ø through fiber times on admission.

acridin orange dye and ordinary light therapeutic use of photosensitizers when he used eosin and white tissue and localization in C., Z. Biol., 1900, 39, 1423) reported the lethal Diamond demonstrated a porphyrin could preferentially degrade tumor implants in a (Diamond, I.; McDonagh, A.F.; Wilson, C.B.; Granelli, S.G.; This result was of administered porphyrins in tumor tissue It was not until 1972, however, reported the effect in 1913 almost Dougherty, T.J.; Grindey, The phototoxic observed Nielsen, S.; Jaenicke, R., Lancet, 1972, 1175). Photosensitizers have been recognized for successfully, when Tappeneir administered porphyrin in man was these two ideas (photodegradation of light to treat skin tumors. In 1903, von effects of a combination of confirmed and extended by recognized in the 1940's. together localization of In 1900, (Rabb, Paramecium. саше <u>و</u>

Inst Cancer Natl. ٦. D.G.; Weishaupt, K.R.; Boyle, 115. ж .. 55,

is used for the treatment and detection of cancer. For detection early stage small tumors, the porphyrin-containing tumor cells much tumors porphyrins for The photoactive dye type therapy t which T.J.; PDT techniques dyes become toxic to the surrounding environment by ., O depend strongly on how well the compound used preferentially is the avoid enondh the allowing ď = the dye to produce toxins which kill the tumor. higher concentration of porphyrins in malignant ligh must single oxygen and oxygen radicals (Dougherty J.H.; Goldfarb, A.; Weishaupt, K.R.; Boyl Skin photosensitivity certain porphyri Because skin retains these chemicals in cancer, photodynamic a strong fluorescence, which contrasts with The irradiating the tumor area with a wavelength of patients tissue, Ø light. Mittleman, A.; Cancer Res., 1976, 38, 3628). (PDT) consists of injecting the patient with reactions, surrounding tissues are exposed to normal PDT with concentrates within the tumor cell. the treatment of weaker fluorescence from the produce surface effect of exposure to sunlight. side For photosensitizers. ţ only known detection. then emit quantities activates porphyrin producing The Kaufman,

ompared ibution thought O The distr distribution of porphyrin drugs in the body . t is injected intravenously, It varies with cell type and porphyrin derivative. with tumor cells is still under investigation. that once the photosensitizer

Fluorescence the interstitial Each porphyrin, then, rapidly within and cells membrane and porphyrin-treated leukemia L1210 cell. into membrane cellular the drug escapes the blood stream and moves regions inside the the plasma cytoplasm. t 0 binds the cell around binds to hydrophobic porphyrin intracellular vesicles the microscopy of diffuses into localization The fluid.

body oligomers linked with ether, and possibly carbon-carbon linkages The main components of Photofrin® are dimers and higher mixing al (Lipson, followed by hydrolysis and precipitation under acidic conditions. Bellnier, D.A.; Wityk, K.E., Adv. Exp. Biol. Med., 1983, 160, of Potter, hematoporphyrin derivative (Dougherty, currently a variety of Hpd thus produced consists of a variety of porphyrins. sulfuric Hematoporphyrin derivative (Hpd) is prepared by Drug Advances, Porphyrin Photosensitization," Plenum weight portion, (Dougherty, The recommended human dosage of Photofrin® is 1-2 mg/kg R.L.; Baldes, E.J.; Olsen, A.M., J. Natl. Cancer Inst., gel "Photodynamic Therapy was partially described by Lipson et В.; is separated into its two main fractions by 3) is the only photosensitizer and D.G.; Weishaupt, K.R.; Henderson, agent of glacial acetic acid the treatment the higher molecular Tsao, PDT efficient Σ.Σ Boyle, D.G.; Weishaupt, K.R., the world for Siegel, more hematoporphyrin with Sephadex LH-20, Photofrin®, a York, 1983, p. Ø R.K.; Photofrin®, is over method (Pandey, This Hpd

Environ. Mass Spectrometry, and Biomed. T.J., Dougherty, 405). 19,

alignant approved porphyrin mixture improvement tissues, activated by penetrating light (>600 nm), photochemically efficient. Although Photofrin®has been for commercialization in Canada, Europe and the United St oligomers, and has the disadvantage that its absorbance a selectively taken up and/or retained in Nev photosensitizer to be clinically useful, a complex the tissue penetration. for is photodynamic therapy for cancer treatment. lacks rapid clearance from tissues, needed are thus for optimized photosensitizers For a

2-(1- e_{6} , monoaspartyl chlorin e_{6} and diaspartyl chlorin e_{6} , were found tissue prior ewed by chlorin Cancer group xcellent T.J., Shaiu, orin There is a need for more efficient, chemically pure, Inst., 1988, 80, 330). With these compounds, the aspartyl Pandey, R.K.; Majchrzycki, D.F.; Smith, K.M.; Dougherty, Important effective photosensitizers in vitro (Roberts, W.G.; series, certain alkyl ether derivatives including Proc. SPIE, 1989, 1065, 104. The aspartyl derivatives of Nelson, J.S.; Smith, K.M., Roberts, M.W., J. Natl efficiency of In pheophorbide, pyropheophorbide and chl Ψ art porphyrin and chlorin derivatives have been revi compounds, hexyloxyethyl)2-des vinyl derivatives were found to be phototoxic, and better localizing porphyrins. parent noted to be responsible for the with compared photosensitizers clearance. to be

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This was attributed to the increased hydrophobicity of the hexyl group and is consistent with studies Evenson on porphyrins with varying polarities (Evenson (Pandey, Cancer, ۲. ${\tt chlorin}_6.$ Br. Dougherty, S.; Riminfton, C.; Moan, J., and pheophorbide-a, pyropheophorbide-K.M.; 53, 65). Smith, D.A.; Photobiol., 1991, J.F.; Sommer, done by

been extended in dihydroxy been reported that the regiospecificity of pyrrole subunits in osmium tetroxide pyropheophorbide-a methylester, have strong absorption in the red region (730 to 750 nm), but, did not show any significant in vivo photosensitizing activity (Kessel, D.; Smith, K.M.; Pandey, R.K.; A.B.; Dougherty, T.J.; Smith, K.M., Bioorg preparation at trimethylester of vic -dihydroxy and keto-bacteriochlorins (Pandey, affected significantly by the presence of Vic Photobiol., Chem. that ţ It has also substituents in the macrocycle(5a). converted has series, by bacteriochlorins, prepared from mesochlorin e₆ ۲, shown methodology · · Photochem. Commun., 1986, 1213, have previously Chang, C.K., Sotiroiu, C.; Wu, reacting with osmium tetroxide can be 491). chlorin e₆ This 1992, 2, ъ, В., Henderson, bacteriochlorin system. and Chem. Lett., Sumlin, the pheophorbide-a oxidation is withdrawing & Med. Shiau,

purpurin-18 Sery, T.W.; Yamamoto, showed that 579 48, Hoober, J.K.;

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strong absorption at 700 nm might be a useful photosensitizer for photodynamic therapy (PDT).

T.J.; absorptions in the visible spectrum can be used to photoactivate strong (760-780 nm) are extremely sensitive to oxygen, which results in thus the a laser is used to excite the bacteriochlorin in a new chromophore Some naturally chlorins (Pandey, lost. (Beems, Smeets, M.F.M.A.; Boehgeim, J.P.J., Photochem. Photobiol., 1987, 46, photosensitizers occurring bacteriochlorins, have previously been reported reducing Dougherty, Ð candidates for use in photodynamic therapy (PDT) where potential effective photosensitizers both in vitro and as in vivo J.A.B.; 639). However, most of the naturally occurring bacteric dyes previously located in targeted (neoplastic) tissues spectroscopic properties of the bacteriochlorins ar rapid oxidation to the chlorin state (640 nm); E.M.; Dubbelman, T.M.A.R.; Lugtenburg, J.; Best, window, thus Smith, K.M., Tetrahedron Lett., 1992, 33, 7815). Shiau, F.Y.; Isaac, M.; Ramaprasad, S.; vivo, oxidation may result in the formation of have been proposed as absorbing laser wavelength the photodynamic efficiency. absorbing outside bacteriochlorins long Further, if Among

meet the cult and both an agent. It has been found that certain cyclic amide derivatives compounds requirements of an improved photodynamic therapeutic porphyrins, including bacteriochlorins and chlorins, have Unfortunately, the preparation of such compounds is diffi increased wavelength and the requisite stability to Such to 30%. 10 e.g. very low, are yields

cyclizing chlorin-The resulting cyclized reaction mixture contains The synthesis route therefore is but requires Further such compounds have reacted a number of products in addition to the cyclic amine, (compound 3 low yield, compound 8) have in the past been prepared by not had optimal hydrophylic-lipophylic balance. 34 significant subsequent purification. because of ester unreacted starting material 3A. 6-N-hexylamide-7-methyl inefficient diazomethane). only

Brief Description of the Drawings

Figure 1 is a schematic equation showing the degeneration of bacteriochlorophyll-a to chlorin.

Figure 2 is a schematic equation showing the synthetic route to compounds 6 and 7 of the invention and their use as intermediates to compound 8.

Figure 3 is a schematic equation showing the synthetic route o cyclic anhydride, compound 1.

Figure 4 is a schematic equation showing the synthetic route to cyclic isoimides and cyclic imide.

Figure 5 is a schematic equation showing the synthetic route to isoimide bacteriochlorins 17 and 18 of the invention.

Figure 6A is a reflection spectroscopy curve showing preferential accumulation of compound 18 in tumors over muscle.

Figure 6B is a curve showing the ratio of tumor over muscle accumulation as represented in Figure 6A.

provided infrared Such compounds ility In accordance with the present invention, there are bacteriochlorin derivatives having ut excited by microwaves, ultrasound, and visible or and photosensitizing compounds. chlorin and fluorescent radiation. þe

traditional areas where compounds having such properties have utility. The compounds, may, for example, be incorporated into a used in purpose substance such as a plastic product, excited with ultrasound, methods All of such novel compounds described herein may be the microwaves or visible light followed by using known detecting emitted radiation to image the product for detecting voids or other flaws in the product.

utility for photosensitizers in the area of photodynamic therapy special Certain of such compounds have detection and treatment of tumors.

More of long wavelength absorbing have PDT tumor site, photosensitizers such as stable bacteriochlorins which accordance with the invention, to make ability to localize in high concentration at the applicable, there is a need

and efficient effective method for preparing such photosensitizers. a need for an Furthermore there is

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therefore 15 compound a chemical of the formula: Ø the invention, accordance with provided which comprises

=0; R_6 may be taken together with R_7 to form =0; R_8 may be taken chemical bond and form =0; R₁₀ may be taken together with R₁₁ ဌ other a polyamine group, -OH, alkyl, carbonyl group, provided that; R_4 may be taken together with and R_{11} may together form a chemical bond; and R_{12} OR₁₃ where amino 0|| =0; and R_4 and R_7 may together form a Ø S пe , Haryl, or о Ч one through R₁₁ are substituted alkyl, a polyether group that if is H, alkyl or NR14; provided or acid group; R_{4} together with Rg to or where R₁₆ group, alkyl; alkyl,

additional the The invention further includes a method for using of the preparation for an intermediate

10

for method ď photosensitizers and long wave length stable

Detailed Description of the Invention

ation of of possible substituents number The invention permits more flexibility in the prepar porphyrin-type compounds than was previously Intermediate compounds may be provided with a on the a and b rings and variable substituents

the R4and groups usually contain 1 through 8 carbon atoms and more commonly aryloxy chain formal substituted and unsubstituted alkyl, alkoxy, alkenyl, aryl, many as 2, of such carbon containing groups may be long The a and b rings may be saturated or unsaturated at contain 1 through 3 carbon atoms. A limited number, i The alkyl, alkoxy, alkenyl, aryl and contain hydrogen, hydroxy, carbon containing groups, e.g.; up to 22 carbon atoms. may positions or groups. aryloxy

with amino ğ substitute sulfo, carboxy, halo, carbon containing groups may be carbonyl, hydroxy, phosphoro, ether substituents. To obtain the compounds of the invention, a substituted or Trans. I, 1973, 2517, to obtain a six membered anhydride ring known Perkin suitable bacteriopurpurin-a containing an anhydride ring before subjecting is converted bacteriochlorin is reacted by methods, as described in Kenner et al., J. Chem. Soc., Ø to obtain ď fused to the macrocyle. For example, bacteriochlorin, bacteriochlorophyll unsubstituted chlorin or

anhydride The carboxylhexyl open the it to the subsequent reactions described herein. amine to 9 obtain 2 to hexyl shown in Figure is then reacted with 1, γ-carboxy-hexylamide 3 The 6 carboxyhexylamide and the $\gamma-$ carboxyhexylamide are then preferred dicyloberylcarbodiimide (DCC), which results the t t carbodiimide where porphyrin diimide which is unstable and immediately invention. ~ in Figure substituents may vary as described herein. separately or together with a the of 7 6 and compound ţ similar the carbodiimide is an isoimid, compounds

detail by The invention may be described in more to the following specific embodiment.

anhydrides into imides, (e.g. such as heating with imidazole at 3 into the corresponding aromatic TOOL conditions, refluxing purpurin-18 methyl ester 1 was used as the starting material. the at (determined by using proton NMR) with \max purpurins with in minor expected, reaction of 1 (Amax 700 nm) with 1-hexylamine following the methods used in converting Leaving tetrahydrofuran establish the reaction a mixture of 2 Ву (705 nm) product. gave decomposition products. temperature for a week gave a mixture of Attempts to convert the amides 2 or anhydride 1 (700 nm), cyclic imide 8 a major corresponding amides in 95% yield as dichloromethane or Initially, in order to a S starting material ratio of 9 to 1 solution in 140°C) mainly imides by and the

desired

any

ot

purpurin-anhydride without formation

of

yield

improved

temperatures, slightly

reaction mixture at various

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similar under in 1,1'-thiocarbonyldiimidazole but purpurin-imide, gave DCC with conditions Replacing (Table 1) reaction

the dicyclohexylurea A proposed mechanism for the formation of the imide ring give now understood that these intermediate species are not stable conditions appropriate dicyclohexylcarbodiimide intermediate this acid to the carbodiimide will an activated carboxylic acid derivative. cyclic isoimides 2 chemistry, accordance with the invention is shown in Figure under basic and tetrapyrrole isoimides of attack the formation In corresponding nucleophilic addition of the carboxylic cyclic imide. example of 0-acylisourea, Intramolecular methanolic KOH, convert to generate

not

are

reaction

6 and

stable and converted to corresponding isoimide analogues

corresponding carbodiimides

afforded

(1:6) in 96% yield. This reaction is precisely the same

described in parent Application Serial No.

except

and

product (12%)

easily

were

which

major product (85%),

anhydride analog 1 as

amides

purpurin

of

Reaction

separated by column chromatography.

and γ -carboxy-hexylamide

6-carboxyhexylamide 2

mixture of

again

a solve

tion

Reac

optimize

imide analogue. Various attempts were then made to

reaction conditions, as summarized in Table 1.

amides 2 and 3 with K-10 clay using CH₂Cl₂ as

mixture of cyclic imide 8 as minor

gave a

carbodiimide and isoimide analogs were prepared converting it to Was Bacteriopurpurin-a 12 S S ζq Spheroides by following the methodology 08/247,866 a substrate and No. U.S. Patent Application Serial n propanol. as the related imide derivative. using bacteriopurpurin-a 12 bacteriochlorin with Related from R.

Both

spectroscopy

and

characterized by proton NMR

isomers were

alone

solvents

ö

Treatment

stronger

DBU with

Interestingly, replacing

8 in 60% yield.

re, gave

bases, such as methanolic KOH or NaOH at room temperatum

the desired purpurin-imide in 85% overall yield (Amax

705nm)

This reaction was repeated several times using individual

isoimide isomers (6 or 7), and produced the desired imi

>80% yield without formation of purpurin-18 methyl

Đ

isoimides (either 6 or 7) with DBU/toluene at 60°C produced imide

with K-10 clay gave mainly the starting material 1.

the isoimide (6 and 7) with various

that the useful end products were inadvertently mischaracterized

as the unstable carbodiimide analogs rather than isoimides.

Separation of the mixture gave pure isomers 6 and 7 with long

wavelength absorptions at Amax 696 and 690nm respectively

ð 14, which to produce 5 and bacteriochlorophyll-a from R-speroides with n-propanol. on reacting with dicyclohexylcarbodiimide is believed derivatives 12a with n-hexylamine gave the amide analogs 13 which ል obtained component) carbodiimide WAS 12a (major corresponding unstable compound 16 and component)

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the compounds with with replace ectively (a) introduce primary- or secondary alkyl ether groups at position <u>@</u> ning g at absorption t O resp various hydrophobic properties is in progress, e.g. increase/decrease the length of alkyl amides by ope and anhydride ring with various amines, and amino acids replace the methylester group (at position -7, ring carbodiimides, analogs, (c) 804 (£=109,000) synthesis of a series of related corresponding isoimides with long wavelength esters or aspartic acid dicyclohexylcarbodiimide with other and Amax 18 796nm 17 (*t*=89,000) of the macrocycle. Currently, the various

experiment, the uptake of bacteriopurpurin 18 in tumor vs. muscle initial gures 6A with erential ging, Stud compound to be useful for PDT and IR ima should have preferential accumulation in tumor. In an was measured by in vivo reflection spectroscopy. From Fi and 6B it can be seen that bacteriopurpurin 18 shows pref accumulation of drug in tumor than muscles (8:1). other related compounds are currently in progress. ď

Experimental

Chemistry:

are GE urchased using and from Aldrich, ACROS Organics and Sigma. Mps were taken instrument using CDCl $_{
m J}$ as solvent. Electronic absorption Commercially available compounds and reagents were NMR spectra were recorded at 300 MHz Fisher-Johns hot plate melting point apparatus were recorded using a Genesis-5 spectrophotometer. uncorrected.

purpurin-imide of preparation intermediate: the New and novel method for carbodiimide isoimide via

solvent and hexylamine solvent The reacted with dicyclohexylcarbodiimide (DCC) (400 mg, 1.75 mmol) was concentrated to 10 ml and left overnight in the refrigerator; (245 mg) was dissolved removed residue reaction mixture separated ot derivatives The The not stable (25 1-hexylamine Spectrophotometry was used to monitor disappearance of KOH (0.5 0.34 give stirred dichloromethane a nitrogen atmosphere with stirring for 12 h. Was at 666 nm. the mixture the The filtrate was concentrated and temperature of j. crystallized from dichloromethane/hexane to mg, and by-product the formation of these compounds ratio reaction mixture was For Were isoimide solution of The dichloromethane (100 ml) was treated with The Ø (200 removed under high vacuum; new peak derivatives 2 (major) and 3 (minor) as ഗ and 7 (in the 220 mg). щg). gel). and -1 dissolved in to be room (245 ø ester purpurin-imide, the mixture of 6 4 a methanolic as of a preparative plates (silica Ŋ carbodiimides yields, respectively (total: The yield was 90% determined a 4 and dicyclohexylurea formed methy1 700 nm and appearance The stirred Was individual isomers and water) was added. mmol) Were (50 ml), intermediate mechanism of and compounds

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705 nm). The mixture was then diluted with dichloromethane (100 ml) and washed with water (3 x 100 ml). The organic layer after new peak of the The syntheses of llica gel 8 was other purpurin-imides 9-11 (see Table 2), the intermediate solated dichloromethane/hexane, and the desired purpurin-imide appropriate eluates were combined. The residue obtained Evaporation crystallized converted into the desired imides in one-pot solvent gave a residue which was chromatographed on a si column (elution with 2% acetone/dichloromethane) Ø carbodiimides and isoimide analogs were not and was monitored by spectrophotometry (appearance of For the dried over anhydrous sodium sulfate. 85% yield (185 mg from 1). was solvent further purification. ... L. evaporating but were obtained

Isoimide derivative of Bacteriopurpurin-a propyl ester (17) a

The and the residue was crystallized from dichloromethane/hexane to obtained from R. spheroides was dissolved in dichloromethane (100 0.05 mmol), of the a new for 24h; vacuum, mmol). give hexylamine derivatives 13 (minor) and 14 (major) spectrophotometry was used to monitor the disappearance peak at 786nm. The solvent was then removed under high The reaction peak at 813nm (due to starting material) and appearance mixture was stirred at room temperature treated with 1-hexylamine (0.2 ml, 0.1 Bacteriopurpurin-a propyl ester 12a (30 mg, mixture in 10 and 90% yields respectively. and was reaction

The solvent removed by The filtrate was concentrated and separated into 0.25 mmol) was concentrated to 10 ml and left overnight in the refrigerator; 72% overall). (25 ml) to 9) found mg, 0.05 mmol) was dissolved in dichloromethane under a nitrogen atmosphere with stirring for 12 h. a by-product was the basis of NMR data, these compounds were preparative plates (silica gel). Yield: (28 mg, (20 individual isomers 17 and 18 (in the ratio of (DCC) isoimide derivatives of bacteriopurpurin-a with dicyclohexylcarbodiimide as dicyclohexylurea formed filtration. <u>د</u>

Spectroscopic Data:

Purpurin-18-N-hexylimide Methyl Ester (8): (Fisher's Nomenclature)

Mp. 221-223°C. UV/Vis: (λ max/nm, ε): 705 (46,000); 647 (12,000); 549 (23,000); 510 (10,000); 483 (8,000); 417 (120,000).

¹H NMR (δ ppm, CDCl₃): 9.63 (s, β-meso H), 9.38 (s, α-meso H), 8.58 (s, δ-meso H), 7.92 (dd, J 19.5, 12.8 Hz, 2a-H), 6.29 (dd, J 19.5 Hz, 2b-H), 6.18 (dd, J 12.8 Hz, 2b'-H), 5.37 (d, J 8.5 Hz, 7-H), 4.48 (t, N-hexylimide-a-CH₂), 4.38 (q, J 8.0 Hz, 8-H), 3.84 (s, 1-meso), 3.62 (q, J 7.5 Hz, 4a-CH₂), 3.56 (s, OMe), 3.34 (s, 1-me), 3.18 (3-Me), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.40 (m, 7a-H), 2.06 (m, 7a'-H), 2.00 (m, N-hexylimide-b,c-CH₂CH₂), 1.74 (d, J 8.0 Hz, 8-Me), 1.65 (t, J 7.2 Hz, 4-b Me), 1.43 (m, N-hexylimide-d,e-CH₂CH₂), 0.46 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -0.08 and -0.17 (each br s, NH). m/z (LRMS): 661 (M+H).

Purpurin-18-15 $^{
m l}$ -hexylisoimide methyl ester (7)

6.31 (dd, J 19.4 Hz, 2b-H), 6.18 (dd, J 12.2 Hz, 2b'-H), 5.26 isoimide-CH $_2$), 3.83 (s, 5-Me), 3.76 (q, J 7.5 Hz, 4a-CH $_2$), 3.58 7b'-H; m, 7a-H; m, 7a'-H; m, hexyl isoimide-b,c- CH_2CH_2 ; 1.78 Hz, 2ahexy1 .58-2.00 .61 (m, 8-H), 4.10 (m, α -meso H), 8.65 (s, δ -meso H), 7.92 (dd, J 19.4, 12.2 OMe), 3.46 (s, 1-Me), 3.28 (3-Me), 2.65 (m, 7b-H), 2 hexylamide-d, e- CH_2CH_2), 0.96 (t. J 7.8 Hz, hexyl isoimide (M + H). 1 H NMR ($^{\delta}$ ppm, CDCl $_{3}$): 9.73 (s, β -meso Hz, 4-b Me), -0.61 and -0.88 (each br s, NH). m/z (LRMS): 661 J 8.5 Hz, 7-H), 4.57 (q, J 8.0 Hz, J 7.2 8.0 Hz, 8-Me), 1.72 (t, 138-139°C. b (q, , g,

Purpurin-18- 13^1 -hexyl isoimide methyl ester (6)

²СН₂; m, isoimide-a-CH₂), 3.81 (s, 5-Me), 3.74 (q, J 7.5 Hz, 4a-CH₂), 3.56 OMe), 3.42 (s, 1-Me), 3.24 (3-Me), 2.65 (m, 7b-H), 2.51-2.00 J 8.5 Hz, 7-H), 4.52 (q, J 8.0 Hz, 8-H), 4.06 (t, hexyl 6.33 (dd, J 19.2 Hz, 2b-H), 6.15 (dd, J 12.5 Hz, 2b'-H), 5.24 Hz, 2a-S, NH) 4-b Me), 1.58 (m, hexyl isoimide-d, e- CH_2CH_2), 0.98 α-meso H), 8.75 (s, δ-meso H), 7.94 (dd, J 19.2, 12.5 7b'-H; m, 7a-H; m, 7a'-H; m, hexylisoimide-b,c-CH₂ 8-Me), 1.6 -0.84 (each br β-meso 142-143°C. ¹H NMR (8 ppm, CDCl₃): 9.74 (s, dicyclohexylisourea- CH_2), 1.76 (d, J 8.0 Hz, Hz, hexylisoimide- $f-CH_3$), -0.66 and (LRMS): 661 (M+H).

Ester Dimethyl Purpurin-18-N-hexylimide-7-N-aspartylamide

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[Fisher's Nomenclature]

8.58 (s, ð-meso H), 7.90 (dd, J 19.8, 12.6 Hz, 2a-H), 6.98 (d, J NMR (b ppm, CDCl₃): 9.60 (s, β -meso H), 9.34 (s, α -meso H), (11,000); 549 (21,000); 510 (9,200); 483 (7,800); 417 (112,000). , 2H 2.84 (m, 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), 1.45 (m, N-hexylimideaspartate-CH₂), 2.64 (m, 7b-H), 2.51 (m, 7b'-H), 2.46 (m, 7a-H), 9.6, aspartate-NHCO), 6.32 (dd, J 19.8 Hz, 2b-H), 6.18 (dd, and 4.38 (43,200); 2.06 (m, 7a'-H), 1.99 (m, N-hexylimide-b,c-CH₂CH₂), 1.75 d,e-CH₂CH₂), 0.96 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -0.38 4a-CH₂), 3.61 (s, OMe), 3.36 (s, 1-Me), 3.16 (3-Me), aspartate-CH), 3.82 (s, 5-Me), 3.69 (s, OMe), 3.64 (q, 8.0 Hz, 8-H), 12.6 Hz, 2b'-H), 5.34 (d, J 8.5 Hz, 7-H), max/nm, ɛ): 705 0.11 (each br s, NH). m/z (LRMS): 791 (M+H). hexylisoimide-a-CH₂), 4.44 (q, JUV/Vis: (λ 218-219°C.

Purpurin-18-N-hexylimide-7-N-Aspartylamide-di-tert-butyl

9.6 Hz, aspartate-NHCO), 6.32 (dd, J 19.4 Hz, 2b-H), 6.21 (dd, J H), 4.41 (t,N-hexylimide- CH_2), 3.99 (m, aspartate-CH), 3.84 (s, (42,800); 3.36 (s, 1-Me), 3.18 8.58 (s, ô-meso H), 7.86 (dd, J 19.4, 12.5 Hz, 2a-H), (11,000); 549 (20,000); 510 (9,000); 483 (7,500); 417 $CDCl_3$): 9.63 (s, β -meso H), 9.37 (s, 12.5 Hz, 2b'-H), 5.33 (d, J 8.5 Hz, 7-H), 4.66 (q, J 705 UV/Vis: (\lambda max/nm, \varepsilon): 5-Me), 3.68 (q, J 7.5 Hz, 4a-CH₂), Mp. 190-192°C. 1H NMR (ô ppm,

2.76 (m, aspartate-CH₂), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.46 aspartate-^t Bu), 1.34 (s, aspartate-^t Bu), 1.14 (m, N-hexylimide-(m, 7a-H), 2.06 (m, 7a'H), 1.92 (m, N-hexylimide-b,c-CH₂CH₂) 1.37 (s, 38 and d,e-CH₂CH₂), 0.95 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -0. reduj 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), (HRMS): ш/2 C₅₁H₆₇N₆O₇: 875.5071. Found 875.5016. Mass: 0.11 (each br s, NH). 1.68 (d, J

Ester-7-aspartylamide-di tert-butyl Ester (11): [Fisher's Nomenclature] Purpurin-18-N-glycylimide-tert-Butyl

2.01 (m, 7a'-H), 1.73 (d, J 7.5 Hz, 8-Me), 1.67 (t, J 7.5 Hz, 4-b .6 (dd, J 12.5 Hz, 2b'-H), 5.27 (d, J 8.5 Hz, 7-H), 5.18 (q, glycine-CH₂), 4.68 (q, J 7.5 Hz, 8-H), 4.38 (m, aspartate-CH), 3.38 (s, 5-Me), 2.79 (m, aspartate-CH₂), 2.66 (m, 7b-H), 2.54 (m, 7b'-H), 2.46 (m, 7a-H), (10,000); 549 (19,000); 510 (8,700); 483 (7,300); 417 (105,000) -meso H) 65 (d, Mp. 138-139°C. UV/Vis: (λ max/nm, ε): 705 (41,300); aspartate-t Bu), 0.10 and -0.04 (each br s, NH). m/2 (LRMS) 1 H NMR (δ ppm, CDCl $_3$): 9.61 (s, eta-meso H), 9.35 (s, lpha-8.56 (s, ô-meso H), 7.88 (dd, J 19.5, 12.6 Hz, 2a-H), 6: 9.5 Hz, aspartate-NHCO), 6.26 (dd, J 19.5 Hz, 2b-H), 6.1 3.64 (q, J 7.5 Hz, 4a-CH₂), 3.35 (s, 1-Me), 3.16 (3-Me), Bu), Me), 1.58 (s, glycine-t Bu), 1.38 (s, aspartate-t

(48,000); 363 (102,000). NMR (CDCl3, & ppm): 9.21 (s, 1H, 5- H), UV/Vis (CH₂Cl₂, Amax, nm): 813 (56,000); 543 (32,000); 408 Bacteriopurpurin-a 17-propyl Ester (12a): [Fisher's Nomenclature] 8.79 (s, 1H, 10-H), 8.62 (s, 1 H, 20-H), 5.14 (d, 1H, $\mathcal{J}=8.0$,

2.14 (m, H, 17a+H), 1.98 (m, H, 17a'-H), 1.81, 1.73 (each d, CO₂CH₂), 3.66 (s, 3H, 12-Me), 3.55 (s, 3H, 2-Me), 3.17 (s, 3H, 8-H), 3.94 (t, 8.2, $CH_2CH_2CH_3$), -0.30 and -0.67 (each br s, Me), 2.73 (m, H, 17b-H), 2.41 (m, 5H, CH₂CH₃ 8.0, 18-Me, 7-Me), 1.11 (t, 3H, J =3-H, 18-H), 4.08 (m, 1H, 2H, 4.30 (m,

(17): Bacteriopurpurin-a-15¹-N-hexylisoimide Nomenclature):

0.97 (t, 5-H), 8.77 (s, (50,400); hexylamide-f- CH_3), 0.88 (t, 3H, J = 8.2, $CH_2CH_2CH_3$), -0.68 CO₂CH₂), 3.69 (s, 3H, 12-Me), 3.58 (s, 3H, 2-Me), 3-Me), 2.62 (m, H, 17b-H), 2.44 (m, 5H, CH_2CH_3 + 7b'-H), 2.14 (m, 6H, 17a-H + hexylisoimide-b,c-CH $_2$ + 3-H, 18-H), 4.11 (m, 3H, 8-H + hexylamide-a-CH₂), 10-H), 8.68 (s, 1H, 20-H), 5.35 (m, 2H, NHCO + 17-H), Mass: LRMS:708(M+H) hexylamide-d, e-CH₂, 1.11 (t, 3H, J = 7.8, 3-b Me), 8.0, 18-Me, 7-Me), 410 (24,500); (89,600). NMR (CDCl₃, δ ppm): 9.21 (s, 1H, λmax: 795 (67,000); 537 1.03 (each br s, 2H, 21, 23-NH). 1.93, 1.84 (each d, 3H, J = 3Н,

isoimide Bacteriopurpurin-a-13¹-N-hexyl

9.38 (s, 1H, 5-H), 8.88 UV/Vis (A max/nm, E): 804 (82,800); 539 (33,200); 409 (59,400); 20-H), 5.46 (m, 1H, NHCO), 360 (94,000). NMR (CDCl3, 6 ppm): d H: 8.73 (s, 1H, 1H, 10-H),

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.93 (each 3.68 (s, d, 3H, J=8.0, 18-Me, 7-Me); 1.57 (m, 4H, hexylisoimide-d,e-CH₂), 3 (m, H, 'H 'E) $e^-f^-{
m CH}_3)$, 8-H) 1Н, 4 3.91 (m, 2H, hexylisoimide-a-CH₂), 4.06 (t, 2H, $co_2 cH_2$), 17b-H), 2.42 (m, 5H, $CH_2CH_2CH_3 + 8a-CH_2 + 7b'-H$), 2.1 17a-H), 2.08 (m, 5H, hexylamide-b, c-CH $_2$ +17a'-H), 2.01, 1 1.12 (t, 3H, J = 7.8, 3-b Me), 0.96 (t, 3H, hexylamid 3H, 12-Me), 3.59 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2. 0.87 (t, 3H, J = 8.2, $CH_2CH_2CH_3$), -0.86 and -1.13 (each 8.0, 17-H), 4.34 (m, 2H, 3-H, 18-H), 4.17 (m,

Biological Studies:

21, 23-NH). Mass: LRMS:708(M+1)

Determination of drug uptake:

Can have scatters pressure tissue (e.g., an experimental mouse tumor) and the light is collected by fixed The noninvasive number of time points after the i.v. injection of an experimental light absorbing compound (e.g., a potential photosensitizer) character of this measurement makes data collection possible at ssue quartz fiber ø Xenon arc lamp and passes through a grating monochromato The absorption spectrum of a compound in living t contact with th at The photo tuned recorded using an instrument and technique which The experiment measures the light which fiber placed in contact with the tissue through the tissue. The light originates in a 5 mm) from the first fiber. bγ The light signal is detected by a photodiode. Hz chopper and then into a 400 μm diameter amplified distal end of this fiber is placed in voltage, converted into a distance (3 to second рe

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at under The chopping spectra examining detected. makes amplifier and synchronously detection synchronous

anesthetized the drug in the The pre-injection to day or hour to hour drift in the total light spectra (pre- and post-injection) are (typically a cuvette and solvents) and the post-injection data taking the ratio of the post-injection spectrum to the same advantages dependence of a wavelength can be thought of as the reference beam a function of wavelength was recorded before second absorption spectrum. The drug was then administered by This in vivo drug absorption spectrum is best the sample beam containing everything in the reference さなり first Ketamine Xylazine i.p. The monochromator expected longest wavelength of the experimental The caused by the presence of light signal which characterizes the instrument. these experiments, the mice were wavelength strength at absorption spectrophotometer. injection spectrum. This ratio offers the recorded. oţ The ratio certainly not influenced by the normalized by dividing the signal drug absorption is negligible signal using either Pentobarbital or injection of the sensitizer. of the lamp, both injection and the light experimental drug. component these double beam day mouse data output

Ø

cacy Effi vivo Bacteriochlorins 17 and 18 using SMT-F Tumor Model - In H Part Efficacy: Photosensitizing

a mouse/tumor model reduction or female mammary vivo via /2 Ha-DD SMT-F tumor, a fast growing spontaneous mouse DBA system. A model system consisted of observing the size DBA/2 HA-DD mice. The tumor line was maintained in subline, transplanted subcutaneously to male mice are readily available and were obtained locally strain. The new photosensitizer was screened in serial transplantation in the same mouse of the tumor

uniform tumor size and allows location of the tumor in the right tumor (1a donor tumor to recipient mouse. This technique provides for relatively injected group overgrown and auxiliary region of the animal within each experimental group. experiments. mouse was (approximately mm cube) were transplanted with a 18 gauge trocar from When tumor reached 4-5 mm in diameter, the animals were potential photosensitizer chosen from th surrounding the tumor was removed with electric clippers of hours after injecting the drug, the and weight (approximately 20 g); small pieces chosen for irradiation, the fur appropriate age Only animals with single tumors were custom-made aluminum holder. the t 0 When mice were both Prior described above. four ø with the placed in

Irradiation Conditions:

d to the guartz total incident dose of 135 J/cm² from a tunable dye laser tune Standard light dose was 75 mW/cm² for 30 min for 2040, Spectra Physics absorption peak. red maximum

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was measured with dye the light to various Laser output interfaced treatment conditions are currently in progress at Was studies light. a microlens deliver a uniform field of Further fiber fitted with

Experimental Procedure:

Following light exposure, the mice were kept in groups of days tumor 80 of both and overlying surrounding skin was monitored daily for non-responsive and supplied with pelleted food gross appearance of early sacrifice of those animals. growth Tumor size and photoillumination unless

The photosensitizer was dissolved in known quantity of Tween 80 (Aldrich) surfactant and diluted by a factor of 10 with saline solution to produce a final Tween 80 concentration of 10-20%. concentration of the solution was determined on the basis of The solution was then filtered through a syringe filter. extinction coefficient value of the photosensitizer Absorption spectra were using a Spectronic Genesis5 spectrophotometer. longest wavelength absorption.

Physics HPLC, connected with C8 reverse phase column, eluted with methanol/water by adjusting the pH to 7.0 using phosphate buffer purity of using the analytical HPLC mice, into drug compounds was ascertained by Before injecting the

Alkylimide derivatives of bacteriochlorins:

N-hexylimide amines derivatives were followed except that various other alkyl of preparation t 0 were substituted for hexylamine. similar Procedures

antitumor The resulting compounds were tested for in vivo 4-6 mm diameter tumors (DBA/2 mice transplanted with SMT-F tumors) were exposed to 75 mW/cm² for 30 minutes to deliver light The tumors were non-palpable and five mice were maximum red Ø to dye laser tuned (135 J/cm²) from a tunable absorption peak. used per group.

large group is The results are in Table 4. The results indicate suppressive effect for isomers where the extended alkyl closest to the amino acid group.

and Part II - In vivo efficacy of bacteriochlorins 17 using Radiation Induced Fibrosarcoma (RIF) Tumor Model.

the and WAS evaluated ä at least every other day thereafter, tumors were measured in orthogonal diameters with an electronic caliper (ultra-Cal Mark automatically recorded, where the tumor volume, V, was calculated group moded) experiment. As shown in Table 3, the animals were treated PDT, for measurement In brief, six mice per for in vivo PDT efficacy using another model (RIF tumor chosen variable doses of light and drug. Beginning 24h after A mixture of bacteriochlorins 17 and 18 was also Each size (4-5 mm) were Boston MA). routinely used in our laboratory. III; Fred V. Fowler Co., with appropriate tumor

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oţ The time for is the longest axis tumor and w is the axis perpendicular to 1. using the formula $v=(1w^2)/2$, where 1

interpolation reached. Was estimated by mm³ 400 then times just before and after to the tumor to 400 mm3 was

response was recorded on the basis of the number of animals which

were found to be tumor free. Appropriate controls were out with tumor-bearing mice which received no

or received light or photosensitizer only.

Merinod No.	Condinons		Yield (%)	(42	
		imide 4	anhydiide 1	starting materials (5 &6)	
	THE refluxing for 4h.	, o	70	25	
2. L	lmidazolc. 1±0°C. 1h	Оесошр	Decomposition products		
Α.	K-10 Clay. CH2Cl2, 24h	10-12	80-85	0	
માં	CH ₂ Cl ₂ , 10 days	15-20	20-25	. 20	
5. DCC with: 6. TCD with: 6. TCD with: 6. TCD with:	ith: a. K-10 Clay, RT b. DBU, RT c. DBU, RT d. KOF/MeOH RT. 10 min rth: a. DBU, RT b. DBU, RT c. DBU, THF 60°C	60 85 10 30	0 0 0 0 0 0	100 100 100	~
ਹ ਹ	KOH/MeOH RT. 10 min	01	0	. 06	

DCC: Dicyclohexyicuroodimide: DBU, 1.8-Diazabicyclo(5.40)undec-7-ene: RT, Room temperature: TCD, 1.1-Thiocarbonyldiimidazole.

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Table 2. Yields of purpurin-imides

H ₃ C H ₃ C H ₀ C N ₀ C	

% yield	85	89	87	78
R	n-hexyl	n-hexyl	n-hexyl	rem-buryl Gly
æ	OMe	Asp-di-methyl ester	Asp-di-terr- butyl ester	Asp-di-terr- butyl ester
Compound No.	80	6	10	11

onse	14	808	4 0 4	803	603	
Tumor Response days	7	100%	\$ 09	100%	80%	
Tun	1-2	100%	% 0 %	100\$	% 0 8	
Timer after Injection hrs		24	24	. 54	24	
Light Dose Rate mW/cm ²		75	30	75	30	
Drug Dose μπο1/kg		0.20	0.20	0.25	0.25	Vile.

ব TABLE

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H₃C
$$\stackrel{\text{H}_3}{\sim}$$
 $\stackrel{\text{H}_3}{\sim}$ $\stackrel{\text{H}_3}{\sim}$

(CH₂)₅CH₃ (CH₂)₅CH₃ (CH₂)₂CH₃ (CH₂)₂CH₃

17. R 19. R 21. R 23. R

MINOR (795 NM)

CO₂C₃H,

I,

H₃C

10 11 11 14 18 11	Comparative	1Ve III VIVO	in vivo Antitumor Activity of Bacteriochlorins	of Bacteriochlorins
Compound	Dose (µmol/kg)	in vivo absorption	Time(h)	vivo Time(h) Tumor response (d) f. • Tumor response (d) f. • Sorbtion Derw injection Tumor response (d) f. • Perw injection Derw injection D
		(Х тах)	and light treatment	1-2 15 30 +
Mixture of 17 and 18	1 1 1	804	======================================	REGROWTH ON DAY 15
20	0.47	804	24	NO RESPONSE
2.2	0.47	804	24	REGROWTH ON DAY 4
Mixture of 23 and 24	Mixture of 0.47 8 23 and 24	804	24	100 40 20

* 4-5 mm diameter tumors were exposed to 75 MW/cm² for 30 min to deliver 135 J/cm² light from a tunable dye laser tuned to the maximum red absorption peak.

d= days.

Non-palpable tumors.

3

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WHAT IS CLAIMED IS

1. A method for the manufacture of an imide derivative of purpurin comprising:

 R_{11} positions may contain at least one group selected from having said macrocycle containing a and b rings which may be saturated to R_{11} positions of the rings and which R_{4} substituted aryloxy a macrocycle with a six membered anhydride ring fused thereto, halo, sulfo, amino and ether, to obtain a purpurin derivative arboxy with reacting hexylamine with a chlorin or bacteriochlorin substituent selected from carboxyl, hydroxy, phosphoro, c carbodiim substituted and group consisting of hydrogen, hydroxy, formal, aryl and reacting the purpurin derivative with a unsubstituted alkyl, alkoxy, alkenyl, may be obtain the imide derivative of purpurin. wherein carbon containing groups or unsaturated at R_4 the and

- 2. The method of Claim 1, wherein the carbodimide is dicycloberylcarbodiimide.
- 3. The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a purpurin imide of the formula:

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where R is selected from the group consisting of OMe and Asp-dimethyl ester and $\rm R_1$ is selected from the group consisting of nhexyl and tert-butyl Gly.

- The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a cyclic imide.
- 5. An imide of purpurin manufactured in accordance with the method of Claim 1.
- 6. An imide of purpurin manufactured in accordance with the method of Claim 2.
- 7. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 3.
- B. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 4.
- 9. A compound of the formula:

= 0 or = NR_{14} ; R_1 is an amino acid group, a polyamine taken together with R_{γ} to form =0; R_{g} may be taken together with =0; and R_{11} may may -0R16' =0; R₆. = NR1, amir alkyl or aryl, or a carbonyl containing group, p together form a chemical bond; and \mathtt{R}_{12} is hydrogen or $\rm R_9$ to form =0; $\rm R_{10}$ may be taken together with $\rm R_{11}$ to form R_4 and R_7 may together form a chemical bond and R_8 and is alkyl; alkyl; provided that if one z is = 0, the other z is alkyl, alkylene, or form substituted alkyl, a polyamine group, R₁₃ to OR₁₃ where that; R₄ may be taken together with R₅ group; R_4 through R_{11} are -H, -OH, a polyether group or R₁₆ is H, group, alkyl,

- The compound of Claim 9 wherein R_{11} and R_{12} are -CH $_3$. 10.
- allyl group. The compound of Claim 9 wherein R_2 is an 11.
- The compound of Claim 9 wherein $R_{\rm 3}$ is an allyl group. 12.
- The compound of Claim 9 having the formula: 13.

where R is normal alkyl of 2 through 12 carbon atoms.

The compound of Claim 9 having the formula:

of Claim 10 wherein R₂ compound 15.

2 through 12

alkyl of

is normal

- The compound of Claim 10 wherein R_3 is O=COR₁₅ 16.
- Claim 15 wherein R₁ The compound of 17.
- is H₃CO₂C-The compound of Claim 16 wherein $m R_2$ 18.
- an isoimide derivative the formula: purpurin comprising reacting a purpurin of the manufacture of method for 19.

=0; R₁₀ ough R11 to form =0; R₆ may be taken together with open substituted may be form a chemical bond and R_{B} and R_{11} may together form a chemical bond; and R_{12} is hydrogen or lower alkyl; provided that may imide to alkyl or R7 t t that; R₄ and form thr if one z is =0, the other z is =NR $_{14}$, with 1-hexylamine carbodi and R_4 alkyl, or group; R4 alkyl, alkylene, $-0R_{16}$, where R_{16} is t t R 6 anhydride ring followed by reaction with a or a carbonyl containing group, provided to form =0; Rg may be taken together with ; 0= polyamine group, or an amino acid ${
m OR}_{13}$ where ${
m R}_{13}$ is alkyl; ${
m R}_{14}$ is purpurin. form with R₁₁ to obtain the isoimide derivative of taken together with R₅ together -0H, taken alkyl, a together

purpurin imide comprising reacting the compound of Claim 9 with alkali metal hydroxide. A method for the manufacture of 20.

comprising metal hydroxide imide reacting the compound of Claim 10 with an alkali purpurin method for the manufacture of

comprising droxide metal hy imide reacting the compound of Claim 15 with an alkali purpurin method for the manufacture of 22. A

comprising droxide É metal imide reacting the compound of Claim 16 with an alkali purpurin method for the manufacture of

comprising metal hydroxide purpurin imide reacting the compound of Claim 17 with an alkali A method for the manufacture of

comprising reacting the compound of Claim 18 with an alkali metal hydroxide imide purpurin A method for the manufacture of 25.

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comprising with alkali of purpurin imide reacting the purpurin derivative from Claim 19 manufacture the method for hydroxide K

A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali hydroxide 27.

ö imide derivative an A method for the manufacture of purpurin comprising:

a chlorin or bacteriochlorin having positions may contain at least one group selected from the group wherein thereto carboxy, halo, sulfo, substituted aryloxy, membered anhydrid ring fused said macrocycle containing a and b rings which may be unsaturated at R_4 to R_{11} positions and which with amino and ether, to obtain a purpurin derivative; aryl and hydrogen, hydroxy, formal, carbon containing groups may be substituted phosphoro, unsubstituted alkyl, alkoxy, alkenyl, selected from carbonyl, hydroxy, reacting hexylamine with six Ø with οf macrocycle consisting

carbodiimide to obtain the imide derivative of purpurin purpurin obtained the reacting

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J.

2.
$$R_2 = CONH(CH_2)_5CH_3$$
. $R_3 = COOH$ (Major) 3. $R_3 = COOH$, $R_2 = CONH(CH_2)_5CH_3$ (Minor)

3.
$$R_3 = COOH$$
, $R_2 = CONH(CH_2)_5CH_3$

3.
$$R_3 = COOH$$
, $R_2 = CONH(CH_2)_5CH_3$ (Min

CH3

Z

z

Ä

ς Έ

 CH_3

INTERMEDIATE

Z Z

I

H₃C

MeOH/KOH

œ

2

 $R = CONH(CH_2)_5CH_3$

ALL AWD SZZZRRSAT L >

CONVERSION OF BACTERIOCHLOROPHYLL-A INTO CHLORIN IN PRESENCE OF LIGHT AND AIR

Bacteriochlorophyll-a , 780 nm

Chlorin, 680 nm

Clay

FIG 3

FORMATION OF ANHYDRIDE RING

L//9

5/7

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T

CH₃ C₂H₅

I

H₃C-

ر با

H₃C

H₂N(CH₂)₅CH₃

0//

 H_3C

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NOCRO MOLES IK

MINZCLE

TUMOR МИЛТЕЗ 105Т ВИЕСТІОН 0066 1300 1000 3300 3000 1200 900 1000

2.5 Micro Moler in EMT6-BalbC

Y NH

z ||

H₃C

Hexyl = -(CH₂)₅CH₃

 \mathcal{O}_{i}

ī

0

Σ H

Pro₂C

Z

Z II

I

AND

Ţ

H₃C-

I

H₃C

,CH₃ ,C₂H₅

I

2

Pro₂C

DCC: 1,3-Dicyclohexylcarbodiimide

FIG. 5

 $R_2 = R_3 = -(CH_2)_5CH_3$

O'

0

, N₂N

Pro₂C

Ĕ

H₃C

Ξ

H₃C

-CH₃

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 $[13. R_2 = CO_2H, R_3 = CONH-hexyl]$ $[14. R_3 = CO_2H, R_2 = CONH-hexyl]$

12. $R_1 = CH_3$ 12a. $R_1 = (CH_2)_2CH_3$

Pro₂C

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-CO₂-C-NHC₆H₁₁, R₃ = -CONH-hexyl H₃C-NC₆H₁₁

15. R₂ = .

16. R₂ = -CO₂-C-NHC₆H₁₁, R₃ = -CONH-hexyl

INTERMEDIATE

NC₆H₁₁

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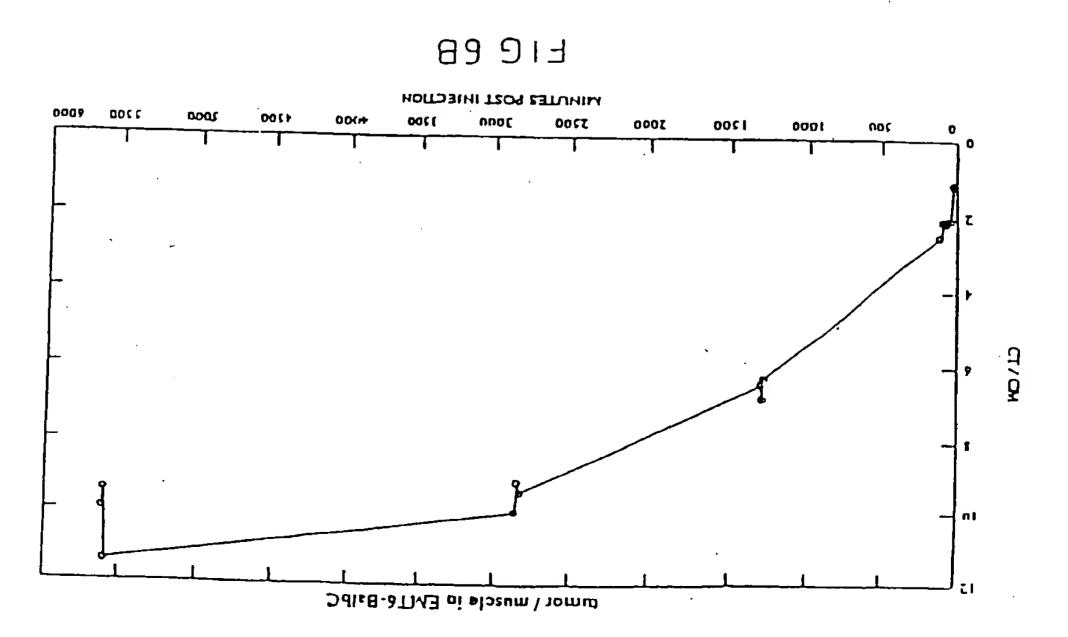
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H₃C,

FIG 6A

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PCT/US97/03891



INTERNATIONAL SEARCH REPORT

International application No. PCT/US97/03891

Date of the actual completion of the international search O2 JUNE 1997 Name and mailing address of the 1SA/US Commissions of Patents and Trademarks Washington, D.C. 20231 Facsimile No. (703) 305-3230 Telephone No. (703) 308 4717 Telephone No. (703) 308 4717	international filing date but later than 'g.' international search Date of many and the search AUS Authorize Authorize Authorize Authorize
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/03891	6860/4	(81) Des
(22) International Filing Date: 7 March 1997 (07.03.97)	7.03.97	CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(30) Priority Data: 08/613,134 8 March 1996 (08.03.96) Not fumished 6 March 1997 (06.03.97)	S n	Published With international search report. With amended claims.
(71) Applicant: HEALTH RESEARCH, INC. [US/US]; Roswell Park Memorial Institute Division, 666 Elm Street, Buffalo, NY 14263 (US).	Roswell Buffalo,	Date of publication of the amended ctaims: 6 November 1997 (06.11.97)
(72) Inventors: PANDEY, Ravindra, K.; 75 Lemay Court, Williamsville, NY 14221 (US). KOZYREV, Andrei, N.; 175 Cambridge Boulevard #1, Amherst, NY 14226 (US). DOUGHERTY, Thomas, J.; 2306 W. Oakfield, Grand Island, NY 14072 (US).	Count, Irei, N.; 6 (US). Grand	
(74) Agent: DUNN, Michael, L.; Dunn & Associates, P.O. Box 96, Newfane, NY 14108 (US).	Box 96,	

(54) Title: SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

(S7) Abstract

Compounds having utility as light absorbing compounds, especially in the area of photodynamic therapy. Such compounds have formula (1), where z is - 0 or NR 14; R14 is alkyl or substituted alkyl, R1 is an amino acid group, a polyamine group, a polyather group or OR 13 where R13 is alkyl; R4 through R11 are -H, -OH, alkyl, alkylene, -OR 16 where R16 is H, alkyl or uryl, or a carbonyl containing group, provided that: R4 may be taken together with R3 to form -O; R6 may be taken together with R3 to form -O; R6 may be taken together with R3 to form -O; R10 may be taken together with R11 to form -O; R10 may be taken together with R11 to form -O; R10 may be taken together form a chemical bond and R3 and R11 may together form a chemical bond; and R12 is hydrogen or lower alkyl; provided that if one z is 0, the other z is -NR14.

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AMENDED CLAIMS

[received by the International Bureau on 9 September 1997 (09.09.97); original claims 1, 2, 9, 11, 17 and 19 amended; original claims 12, 15, 16, 18, 22, 23 and 25 cancelled; remaining claims unchanged (6 pages)]

 A method for the manufacture of an imide derivative of purpurin comprising: orin having and which R₄ saturated selected from substituted aryloxy carboxy, derivative; thereto with and reacting the purpurin derivative with a carbodismide substituted and fused selected from carboxyl, hydroxy, phosphoro, тау ре reacting hexylamine with a chlorin or bacteriochl obtain a purpurin the rings formyl, aryl group six membered anhydride ring rings which alkenyl, R₁₁ positions may contain at least one group consisting of hydrogen, hydroxy, þe at R_4 to R_{11} positions of may purpurin unsubstituted alkyl, alkoxy, carbon containing groups and b ether, to obtain the imide derivative of Ø containing amino and Ø macrocycle with said macrocycle unsaturated sulto, substituent wherein halo, and

- The method of Claim 1, wherein the carbodismide is dicyclohexylcarbodismide.
- obtain derivative alkali metal hydroxide to imide the wherein ٦, the formula: Claim reacted with an o ţ imide of method purpurin further . m

AMENDED SHEET (ARTICLE 19)

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where R is selected from the group consisting of OMe and Asp-dimethyl ester and $R_{\rm l}$ is selected from the group consisting of n-hexyl and tert-butyl Gly.

- 4. The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a cyclic imide.
- 5. An imide of purpurin manufactured in accordance with the method of Claim 1.
- 6. An imide of purpurin manufactured in accordance with the method of Claim 2.
- 7. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 3.
- 8. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 4.
- . A compound of the formula:

40 Amended Sheet (article 19)

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a polyamine acid uted with ether substituents, provided that; \mathtt{R}_4 may be taken together with amino and group, alkenyl chemical bond and $R_{f 1}$ and $R_{f 1}$ may together form a chemical bond; form to form =0; R_6 may be taken together with R_7 to form =0; amino þe containing together with R_{11} to form =0; and R_4 and R_7 may together one is alkyl; alkoxy substit taken together with \mathtt{R}_{9} to form =0; \mathtt{R}_{10} may carboxy, halo, sulfo, is an amino acid group, alkyl; provided that if or alkyl, substituted alkyl, a polyamine group, polyether group or OR_{13} where R_{13} þe carbonyl may group; R_4 through R_{11} are -H, -OH, groups Ø alkylene, aryl, or aryloxy, or carbonyl, hydroxy, phosphoro, NR14; R1 carbon containing and R₁₂ is hydrogen or lower 0, the other z is = NR_{14} . or = 0 Ø wherein 7

- The compound of Claim 9 wherein $R_{
 m 11}$ and $R_{
 m 12}$ are -C 10.
- The compound of Claim 9 wherein R_9 is $-COCH_3$. 11.
- The compound of Claim 9 having the formula: 13.

2 through 12 carbon atoms. alkyl of is normal

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compound of Claim 9 having the formula 14.

2 through 12 carbon atoms. where R is normal alkyl of

- is H₃CO₂C-9 wherein R_1 The compound of Claim 17.
- isoimide derivati a purpurin of the formula: an method for the manufacture of purpurin comprising reacting 4 19.

AMENDED SHEET (ARTICLE 19)

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polyamine tuted with group, amino and gether with form =0; R_B ; puoq alkenyl anhydride 2 is =0 form R14 amino obtain containing chemical bond and $R_{f B}$ and $R_{f 1l}$ may together form a chemical may be R₇ may together alkyl; and R₁₂ is hydrogen or lower alkyl; provided that if one alkoxy, substituted alkyl, a polyamine group, or an open the carboxy, halo, sulfo, ether substituents provided that; \mathtt{R}_4 may be taken to substi group, 40 taken together with R_9 to form =0; R_{10} ìs form =0; R_6 may be taken together with R_7 carbodiimide are -H, -OH, alkyl, R13 is =0 or NR_{14} ; R_1 is an amino acid may be carbonyl the other z is =NR $_{14}$, with 1-hexylamine to group or OR₁₃ where together with R_{11} to form =0; and R_4 and groups alkylene, aryl, or aryloxy, or a with a phosphoro, purpurin. containing reaction group; R₄ through R₁₁ isoimide derivative of polyether carbonyl, hydroxy, followed by wherein carbon alkyl, or wherein z đ pe

- comprising hydroxide method for the manufacture of purpurin imide reacting the compound of Claim 9 with alkali metal
- comprising hydroxide alkali metal purpurin imide Claim 10 with an 21. A method for the manufacture of reacting the compound of
- comprising hydroxide reacting the compound of Claim 17 with an alkali metal purpurin imide A method for the manufacture of
- comprising kali al method for the manufacture of purpurin imide reacting the purpurin derivative from Claim 19 26.
- comprising kali with al 27. A method for the manufacture of purpurin imide the purpurin derivative from Claim 19 reacting

AMENDED SHEET (ARTICLE 19)

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ö derivati imide an of manufacture the method for purpurin comprising K 28.

and aryl and aryloxy, wherein positions may contain at least one group selected from the group reacting hexylamine with a chlorin or bacteriochlorin having carboxy, halo, sulfo, substituen formal, substituted fused рe carbon containing groups may be substituted with a which amino and ether, to obtain a purpurin derivative; and which may ring and anhydrid selected from carbonyl, hydroxy, phosphoro, rings positions unsubstituted alkyl, alkoxy, alkenyl, consisting of hydrogen, hydroxy, and b membered R11 a macrocycle containing 40 six Ø macrocycle with at unsaturated

with of purpurin derivative purpurin carbodiimide to obtain the imide obtained the

AMENDED SHEET (ARTICLE 19)

VERSION*

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ventors: PANDEY, Ravindra, K.; 75 Lemay Court, Williamsville, NY 14221 (US). KOZYREV, Andrei, N.; 175 Cambridge Boulevard #1, Amherst, NY 14226 (US). DOUGHERTY, Thomas, J.; 2306 W. Oakfield, Grand Island, NY 14072 (US).

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(72) Inventors:

(74) Agent: DUNN, Michael, L.; Dunn & Associates, P.O. Box 96, Newfane, NY 14108 (US).

(54) Tille: SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

(57) Abstract

Compounds having utility as light absorbing compounds, especially in the area of photodynamic therapy. Such compounds have formula (I), where z is = 0 or NR14; R14 is alkyl or substituted alkyl, R₁ is an amino acid group, a polyamine group, a polycther group or OR₁₃ where R₁₃ is alkyl; R₄ through R₁₁ are -H, -OH, alkyl, alkylene, -OR₁₆ where R₁₆ is H, alkyl or aryl, or a carbonyl containing group, provided that: R4 may be taken together with R₂ to form -O; R₆ may be taken together with R₂ to form -O; R₈ may be taken together with R₂ to form -O; R₈ may be taken together with R₂ to form -O; and R4 and R3 may together form a chemical bond and R8 and R11 may together form a chemical bond; and R12 is hydrogen or lower alkyl; provided that if one z is 0, the other z is -O; R 10 may be taken together with R11 to form

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* (Referred to in PCT Gazette No. 54/1997, Section 11)

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AND SYNTHESIS OF ISOIMIDE OF CHLORINS AND BACTERIOCHLORINS THEIR USE FOR DIAGNOSIS AND TREATMENT OF CANCER

Background of the Invention

cancer employing analogs porphyrin related compounds and more particularly certain and diagnosis IR imaging and photodynamic therapy This invention relates to treatment chlorins and bacteriochlorins. through the use of

and Most and/or these The growth or nutrient uptake, cells concentrations fluoresce when activated by light of a specific wavelength. tissues. cells retain retention time is not dependent on whether or not the are chemicals which kill for longer durations than surrounding normal tissues substances in higher some premalignant synthesizing DNA or undergoing cell photochemically active Photosensitizers and malignant

irradiation have failed. In this new therapy, patients are given being optic probes have cancer energy been combined in a procedure known as photodynamic therapy (PDT) intravenous injections of a photodynamic drug that accumulates ated PDT has emerged as one of the most promising strategies in cancer cells in much higher concentrations than in normal . 1.S surgery and high activ PDT then sensitive drugs, lasers and fiber İS detection). drug chemotherapy, (photosensitizing) (including cancer increasingly used where photodynamic treatment The

with too frail to tolerate the stress of major surgery, chemotherapy or high energy necessitate hospital any number treatment of dye in cancer that contraindicated cancer which performed laser beam directed to photodynamic therapy, or allows selective malignant tissues due to preferential retention of that too old cancer already been established requires just local anesthesia and does not is not can be superficial malignancy, PDT may be curative an important form of People who are įţ it e.g. therapies and it patient, radiation may be helped by ಥ advantages, ģ single cells through fiber optics. PDT is cells and it has the cancer many additional ם עס cancer admission.

that tissue and localization in of an This result was first tumor tissue recognized in the 1940's. It was not until 1972, however, degrade tumor implants acridin orange dye and ordinary eosin and I.; McDonagh, A.F.; Wilson, C.B.; Granelli, In 1900, (Rabb, C., Z. Biol., 1900, 39, 1423) reported the observed in 1913. The phototoxic effect Photosensitizers have been recognized for almost and extended by Dougherty, T.J.; Grindey, reported Diamond therapeutic use of photosensitizers when he used Jaenicke, R., Lancet, 1972, 1175). administered porphyrins in when Tappeneir porphyrin in man was these two ideas (photodegradation of tumors) came together successfully, preferentially vov tumors. In 1903, effects of a combination of skin that a porphyrin could of treat Paramecium. (Diamond, administered localization .; .; t 0 confirmed

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R.; Weishaupt, K.R.; Boyle, D.G.; J. Natl. Cancer Inst., 1975, 55, 115.

cells avoid etection (PDT) consists of injecting the patient with a photoactive dye tumors the much concentrates within the tumor cell. Skin photosensitivity is the in type The porphyrins therapy which PDT techniques Boyle, D.; entially n enough environment weaker fluorescence from the normal tissue, allowing M and irradiating the tumor area with a wavelength of light tumor. The higher concentration of porphyrins in malignant early stage small tumors, the porphyrin-containing tum detection. For the treatment of cancer, photodynamic oxygen radicals (Doughert) strongly on how well the compound used prefer only known side effect of PDT with certain porphyr MUS photosensitizers. Because skin retains these chemicals i contrasts with patients activates the dye to produce toxins which kill the J.H.; Goldfarb, A.; Weishaupt, K.R.; is used for the treatment and detection of cancer. toxic to the surrounding Mittleman, A.; Cancer Res., 1976, 38, 3628). surrounding tissues are exposed to light. reactions, then emit a strong fluorescence, which surface oxygen and produce dyes become exposure to sunlight. single ţ t quantities porphyrin producing depend

The distribution of porphyrin drugs in the body compared with tumor cells is still under investigation. The distribution varies with cell type and porphyrin derivative. It is thought that once the photosensitizer is injected intravenously, some of

escapes the blood stream and moves into the interstitial Fluorescence and slowly within diffuses into the cell cytoplasm. Each porphyrin, then, The porphyrin binds to the cellular membrane and cell. porphyrin-treated leukemia L1210 membrane binds to hydrophobic regions inside the plasma the vesicles around of localization intracellular the drug

currently being Sephadex LH-20, the higher molecular weight portion, called separated into its two main fractions by gel filtration oligomers linked with ether, and possibly carbon-carbon linkages Cancer Inst., 1961, 26 conditions a variety of porphyrins. a variety of Photofrin®, a hematoporphyrin derivative (Dougherty, sulfuric Drug Advances, Porphyrin Photosensitization," Plenum Hematoporphyrin derivative (Hpd) is prepared by a more efficient PDT agent (Dougherty, McReynolds, dimers acidic Boyle, D.G.; Weishaupt, K.R., "Photodynamic Therapy e t Bellnier, D.A.; Wityk, K.E., Adv. Exp. Biol. Med., York, 1983, p. 3) is the only photosensitizer and Lipson Photofrin® are of Photofrin® is precipitation under of Henderson, glacial acetic acid over the world for the treatment A.M., J. Natl. partially described by Tsao, Hpd thus produced consists of Weishaupt, K.R.; Siegel, M.M.; of recommended human dosage The main components followed by hydrolysis and E.J.; Olsen, hematoporphyrin with Vas R.K.; Photofrin®, is method 18

1990 Spectrometry, Biomed. and Environ. Mass Т.Ј., Dougherty, 405).

for commercialization in Canada, Europe and the United States, it malignant approved of orphyrin must at 630 xture , (mr it photochemically efficient. Although Photofrin® has been complex mi improve oligomers, and has the disadvantage that its absorbance tissues, activated by penetrating light (>600 photosensitizer to be clinically useful, non-toxic, selectively taken up and/or retained are thus needed for the is not optimized for tissue penetration. Ø lacks rapid clearance from tissues, is photodynamic therapy for cancer treatment. photosensitizers ø

less T.J., e₆, monoaspartyl chlorin e₆ and diaspartyl chlorin e₆, were found excellent chlorin Cancer group tissue methyl iewed by Important prior Shaiu, clearance. In pheophorbide, pyropheophorbide and chlorin There is a need for more efficient, chemically pure, Inst., 1988, 80, 330). With these compounds, the aspartyl ק to be effective photosensitizers in vitro (Roberts, W.G.; Proc. SPIE, 1989, 1065, 104. The aspartyl derivatives of Doughert F.Y.; Nelson, J.S.; Smith, K.M., Roberts, M.W., J. Natl noted to be responsible for the efficiency of series, certain alkyl ether derivatives includin art porphyrin and chlorin derivatives have been rev compounds, hexyloxyethyl)2-des vinyl derivatives were found to be Pandey, R.K.; Majchrzycki, D.F.; Smith, K.M.; phototoxic, and better localizing porphyrins. with parent compared photosensitizers

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This was attributed to the increased (Pandey, Cancer, hydrophobicity of the hexyl group and is consistent with done by Evenson on porphyrins with varying polarities T.J and chlorin₆. Dougherty, ц , Moan, pheophorbide-a, pyropheophorbide-K.M.; .; : Riminfton, 53, 65). Smith, s.; Photobiol., 1991, D.A.; J.F.; Sommer, 483).

vic dihydroxy been reported that the regiospecificity of pyrrole subunits in osmium tetroxide pyropheophorbide-a methylester, have strong absorption in the red chlorins, extended region (730 to 750 nm), but, did not show any significant in preparation photosensitizing activity (Kessel, D.; Smith, K.M.; Pandey, Soc., significantly by the presence of Photobiol., Chem. been that reacting with osmium tetroxide can be converted to also Smith, series of vic -dihydroxy and keto-bacteriochlorins substituents in the macrocycle(5a). bacteriochlorin system. This methodology has ρλ bacteriochlorins, prepared from mesochlorin $\mathbf{e}_{\mathbf{6}}$ shown W., J. It has T.J.; series, Photochem. 1213, have previously 491). C.K., Sotirolu, C.; Wu, A.B.; Dougherty, the pheophorbide-a and chlorin e 1992, 2, В., F.Y.; Henderson, affected Sumlin, & Med. Chem. Lett., Commun., 1986, j.s Shiau, F.Y.; Chang, withdrawing Shaiu,

579 showed that purpurin-18 Yamamoto, T.W.; Sery, 48, J.K.; Photobiol., 1988, Hoober,

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strong absorption at 700 nm might be a useful photosensitizer for photodynamic therapy (PDT).

strong absorptions in the visible spectrum can be used to photoactivate useful occurring bacteriochlorins, have previously been reported as R.K.; Shiau, F.Y.; Isaac, M.; Ramaprasad, S.; Dougherty, T.J.; (760-780 nm) are extremely sensitive to oxygen, which results in Some naturally thus the Further, if a laser is used to excite the bacteriochlorin in vivo, oxidation may result in the formation of a new chromophore (Pandey, chlorins M.F.M.A.; Boehgeim, J.P.J., Photochem. Photobiol., 1987, 46, (Beems lost. photosensitizers Smeets reducing a where potential effective photosensitizers both in vitro and as in vivo E.M.; Dubbelman, T.M.A.R.; Lugtenburg, J.; Best, J.A.B.; 639). However, most of the naturally occurring bacterio spectroscopic properties of the bacteriochlorins ar dyes previously located in targeted (neoplastic) tissues oxidation to the chlorin state (640 nm); (PDT) Smith, K.M., Tetrahedron Lett., 1992, 33, 7815). thus for use in photodynamic therapy as absorbing window, have been proposed the laser wavelength photodynamic efficiency. outside bacteriochlorins long Among candidates absorbing rapid

It has been found that certain cyclic amide derivatives of both an Unfortunately, the preparation of such compounds is difficult and meet the agent. porphyrins, including bacteriochlorins and chlorins, have requirements of an improved photodynamic therapeutic componuç increased wavelength and the requisite stability to Such 30%. ţ 10 e.g. low, very are yields

compound 8) have in the past been prepared by cyclizing chlorinbut requires especially Further such compounds have synthesis route therefore reacted mixture amine, ്ന low yield, The resulting cyclized reaction (compound a number of products in addition to the cyclic not had optimal hydrophylic-lipophylic balance. 34 The oţ significant subsequent purification. 6-N-hexylamide-7-methyl ester because starting material 3A. inefficient diazomethane). unreacted

Brief Description of the Drawings

Figure 1 is a schematic equation showing the degeneration of bacteriochlorophyll-a to chlorin.

Figure 2 is a schematic equation showing the synthetic route to compounds 6 and 7 of the invention and their use as intermediates to compound 8.

Figure 3 is a schematic equation showing the synthetic route to cyclic anhydride, compound 1.

Figure 4 is a schematic equation showing the synthetic route to cyclic isoimides and cyclic imide.

Figure 5 is a schematic equation showing the synthetic route to isoimide bacteriochlorins 17 and 18 of the invention.

Figure 6A is a reflection spectroscopy curve showing preferential accumulation of compound 18 in tumors over muscle.

Figure 6B is a curve showing the ratio of tumor over muscle accumulation as represented in Figure 6A.

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Brief Description of the Invention

In accordance with the present invention, there are provided new chlorin and bacteriochlorin derivatives having utility as fluorescent and photosensitizing compounds. Such compounds may be excited by microwaves, ultrasound, and visible or infrared radiation.

All of such novel compounds described herein may be used in traditional areas where compounds having such properties have utility. The compounds, may, for example, be incorporated into a substance such as a plastic product, excited with ultrasound, microwaves or visible light followed by using known methods for detecting emitted radiation to image the product for the purpose of detecting voids or other flaws in the product.

Certain of such compounds have special utility as photosensitizers in the area of photodynamic therapy for the detection and treatment of tumors.

In accordance with the invention, to make PDT more applicable, there is a need of long wavelength absorbing photosensitizers such as stable bacteriochlorins which have the ability to localize in high concentration at the tumor site.

Furthermore there is a need for an efficient and coeffective method for preparing such photosensitizers.

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In accordance with the invention, a compound is therefore provided which comprises a chemical of the formula:

alkylene, and \mathtt{R}_{11} may together form a chemical bond; and \mathtt{R}_{12} is hydrogen chemical bond form =0; R_8 may be a polyamine group, =0; $R_{
m 10}$ may be taken together -H, -OH, alkyl, acid a carbonyl with OR₁₃ where amino together R_{γ} may together form תמ $^{-0R}_{16}$, where R_{16} is H, alkyl or aryl, or =O; R₆ may be taken together with R₇ to . S or group, provided that; R4 may be taken acid group; R_4 through R_{11} are alkyl, polyether group alkyl; provided that if substituted form and or Ø 0 or with R₉ group, alkyl, together

The invention further includes a method for using the above compound as an intermediate for the preparation of additional

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for method Ø and photosensitizers stable length preparation. wave long

Detailed Description of the Invention

The invention permits more flexibility in the preparation of possible number substit previously ø provided with variable Vas and than and b rings þe compounds may compounds Ø the porphyrin-type נס Intermediate substituents

and 9 commonly aryloxy chain the formal aryl, long and b rings may be saturated or unsaturated at aryl and groups usually contain 1 through 8 carbon atoms and more alkenyl, contain 1 through 3 carbon atoms. A limited number, carbon containing groups, e.g., up to 22 carbon atoms. may be hydroxy, The alkyl, alkoxy, alkenyl, substituted and unsubstituted alkyl, alkoxy, 2, of such carbon containing groups contain hydrogen, may or groups. positions aryloxy many as

with amino substituted sulfo, carboxy, halo, may be groups phosphoro, containing carbonyl, hydroxy, ether substituents. carbon

known Perkin suitable ide ring containing an anhydride ring before subjecting substituted or unsubstituted chlorin or bacteriochlorin is reacted by conver Trans. I, 1973, 2517, to obtain a six membered anhydr described in Kenner et al., J. Chem. Soc. obtain To obtain the compounds of the invention, a . . to Ø example, bacteriochlorin, bacteriochlorophyll For the macrocyle. bacteriopurpurin-a ช fused to methods,

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The anhydride anhydride amide carboxylhexyl open the described herein. to 9 2 to obtain amine it to the subsequent reactions hexyl shown in Figure with 1, γ-carboxy-hexylamide 3 is then reacted

The 6 carboxyhexylamide and the $\gamma ext{-carboxyhexylamide}$ are then preferred converts which results carbodiimide porphyrin diimide which is unstable and immediately where compound of the invention. is dicyloberylcarbodiimide (DCC), N Figure substituents may vary as described herein. separately or together with a 'n ~ and ø ţ an isoimid, the similar carbod!imide compounds reacted

by reference invention may be described in more detail to the following specific embodiment.

anhydrides into imides, (e.g. such as heating with imidazole at temperature for a week gave a mixture of purpurins with cyclic into the corresponding amide refluxing gave starting material Leaving the ratio of 9 to 1 (determined by using proton NMR) with Amax anhydride 1 (700 nm), cyclic imide 8 (705 nm) in minor expected, reaction of 1 (Amax 700 nm) with 1-hexylamine and converting tetrahydrofuran establish the reaction corresponding amides in 95% yield as a mixture of 2 Ву major product. decomposition products. imides by following the methods used in or 3 purpurin-18 methyl ester 1 was used as the nm. Attempts to convert the amides 2 dichloromethane or Ø Initially, in order to and the starting material as gave mainly solution in

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the as a solvent again not of and easily purpurin amides amide ction imide analogue. Various attempts were then made to optimize slightly improved are (12%) Rea any 2 and γ -carboxy-hexy] DCC afforded corresponding carbodismides which product which purpurin-anhydride without formation of reaction conditions, as summarized in Table 1. separated by column chromatography. Reaction of as major product (85%), and 3 with K-10 clay using $\mathrm{CH_2Cl}_2$ as minor reaction mixture at various temperatures, mixture of cyclic imide 8 (a mixture of 6-carboxyhexylamide anhydride analog 1 of amides 2

6 and 7 reaction that the useful end products were inadvertently mischaracterized except than isoimides stable and converted to corresponding isoimide analogues described in parent Application Serial No. 08/613,134 (1:6) in 96% yield. This reaction is precisely the same as the unstable carbodiimide analogs rather

Both with long spectroscopy. wavelength absorptions at Amax 696 and 690nm respectively. 7 and characterized by proton NMR and mass 9 Separation of the mixture gave pure isomers were

alone or Treatment of solvents with K-10 clay gave mainly the starting material 1. Refluxing the isoimide (6 and 7) with various

isoimides (either 6 or 7) with DBU/toluene at 60°C produced imide stronger Interestingly, replacing DBU with 8 in 60% yield.

bases, such as methanolic KOH or NaOH at room temperature, gave 705nm), the desired purpurin-imide in 85% overall yield (Amax

reaction was repeated several times using individual

in ester imide yield without formation of purpurin-18 methyl isoimide isomers (6 or 7), and produced the desired

similar lower under Ė 1,1'-thiocarbonyldiimidazole but purpurin-imide, gave with conditions DCC Replacing reaction (Table 1)

with W111 dicyclohexylurea. In brief give ring attack under basic conditions intermediate this the imide addition of the carboxylic acid to the carbodismide will derivative. now understood that these intermediate species are not isoimides chemistry, in Figure of and an activated carboxylic acid dicyclohexylcarbodiimide formation cyclic In tetrapyrrole shown isoimides of proposed mechanism for the is the formation invention nucleophilic corresponding generate cyclic imide. the appropriate example of with 0-acylisourea, Intramolecular methanolic KOH to accordance

of analogs were prepared reaction converting Was 12 ζ Bacteriopurpurin-a from R. Spheroides by following the methodology 08/247,866 substrate and and isoimide No. ๙ U.S. Patent Application Serial bacteriochlorin with n propanol 8 the related imide derivative. using bacteriopurpurin-a 12 carbodiimide Related

14, which produce 15 (minor treatment t C and bacteriochlorophyll-a from R-speroides with n-propanol. derivatives reacting with dicyclohexylcarbodiimide is believed 13 which δ analogs obtained carbodiimide component) 12a with n-hexylamine gave the amide Was (major 128 corresponding unstable compound 16 and Another component)

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absorption at Amax , (a) to increase/decrease the length of alkyl amides by opening the , (b) to compounds with D) with respectively. (q) and various hydrophobic properties is in progress, e.g. anhydride ring with various amines, and amino acids replace the methylester group (at position -7, ring ţ groups at po analogs, (c) carbodiimides, and Amax 18 804 (£=109,000) series of related corresponding isoimides with long wavelength ether secondary alkyl dicyclohexylcarbodiimide with other or aspartic acid Currently, the synthesis of a introduce primary- or (6=89,000) esters 796nm 17

muscle igures 6A and 6B it can be seen that bacteriopurpurin 18 shows preferential an initial with imaging, Studies was measured by in vivo reflection spectroscopy. From F Ι'n experiment, the uptake of bacteriopurpurin 18 in tumor and IR accumulation of drug in tumor than muscles (8:1). other related compounds are currently in progress. should have preferential accumulation in tumor. compound to be useful for PDT

Experimental

S E purchased using and r c taken instrument using CDCl₃ as solvent. Electronic absorptio Commercially available compounds and reagents were spectra were recorded at 300 MHz hot plate melting point apparatus from Aldrich, ACROS Organics and Sigma. Mps were were recorded using a Genesis-5 spectrophotometer NMR Fisher-Johns uncorrected.

purpurin-imide preparation of intermediate: New and novel method for isoimide via carbodiimide

ij. The solvent crystallized from dichloromethane/hexane to give hexylamine and The solvent mg, 1.75 mmol) into The mechanism of the formation of these compounds is shown in Figs. 2 of Œ (2 ml, purpurin-imide, the mixture of 6 and 7 (245 mg) was dissolved a methanolic solution of KOH (0.5 mg/10 the preparation mmo]) removed under high vacuum, and the residue yields, respectively (total: 220 mg). The reaction mixture separated 9 o f compounds were determined to be isoimide derivatives stirred for derivatives 2 (major) and 3 (minor) as a mixture in 84 dichloromethane (100 ml) was treated with 1-hexylamine Spectrophotometry was used to monitor disappearance of (25 structure 0.34 0.34 mmol) was dissolved in dichloromethane 700 nm and appearance of a new peak at 666 nm. under a nitrogen atmosphere with stirring for 12 h. was concentrated to 10 ml and left overnight in the K A S temperature reacted with dicyclohexylcarbodiimide (DCC) (400 filtrate was concentrated and not , pm 5 (in the ratio of dicyclohexylurea formed as a by-product The reaction mixture was For were The (200 ഗ The yield was 90% (245 mg). gel). intermediate carbodiimides 4 and room ester preparative plates (silica at isomers 4 and Purpurin-18 methyl stirred The and added. (50 ml), individual

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new peak romethane and washed with water $(3 \times 100 \text{ ml})$. The organic layer Evaporation of the The from after silica gel syntheses of 8 WAS 9-11 (see Table 2), the intermediate solated appropriate eluates were combined. The residue obtained dichloromethane/hexane, and the desired purpurin-imide and was monitored by spectrophotometry (appearance of a The mixture was then diluted with dichlo column (elution with 2% acetone/dichloromethane) converted into the desired imides in one-pot solvent gave a residue which was chromatographed on a carbodiimides and isoimide analogs were not For the dried over anhydrous sodium sulfate. in 85% yield (185 mg from 1). Was solvent purpurin-imides the further purification. at 705 nm). evaporating (100 ml) Were obtained but

(11) ester Isoimide derivative of Bacteriopurpurin-a propyl

(100 The 24h; and the residue was crystallized from dichloromethane/hexane to of the a new Vacuum, mmol) obtained from R. spheroides was dissolved in dichloromethane or mmol). (major) 0.05 spectrophotometry was used to monitor the disappearance 786nm. The solvent was then removed under high The reaction peak at 813nm (due to starting material) and appearance reaction mixture was stirred at room temperature 0.1 (30 mg, and 14 m), ml) and was treated with 1-hexylamine (0.2 Bacteriopurpurin-a propyl ester 12a give hexylamine derivatives 13 (minor) mixture in 10 and 90% yields respectively. peak at

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was concentrated to 10 ml and left overnight in the refrigerator; ml) separated found (25 1 to mg, 72% reacted with dicyclohexylcarbodiimide (DCC) (50 mg, 0.05 mmol) was dissolved in dichloromethane under a nitrogen atmosphere with stirring for 12 h. Was were concentrated and isomers 17 and 18 (in the ratio of preparative plates (silica gel). Yield: (28 by-product On the basis of NMR data, these compounds isoimide derivatives of bacteriopurpurin-a was a S filtrate formed dicyclohexylurea individual (30 mg,

Spectroscopic Data:

: (8) Ester Methyl Purpurin-18-N-hexylimide Nomenclature]

7-H), 4.48 (t, N-hexylimide-a-CH $_2$), 4.38 (g, J 8.0 Hz, 8-H), 3.84 (s, 5-Me), 3.62 (q, J 7.5 Hz, 4a-CH₂), 3.56 (s, OMe), 3.34 (s, 1-(g 5.37 (d, J.8.5 Hz (12,000); 549 (23,000); 510 (10,000); 483 (8,000); 417 (120,000) hexylimide-d,e- $\mathrm{CH_2CH_2}$), 0.46 (t, J 7.8 Hz, N-hexylimide-f- $\mathrm{CH_3}$), Me), 3.18 (3-Me), 2.65 (m, 7b-H), 2.51 (m, 7b'-H), 2.40 (m, (46,000); H), 2.06 (m, 7a'-H), 2.00 (m, N-hexylimide-b,c-CH₂CH₂), m/z (LRMS): 661 (M+H) NMR (δ ppm, CDCl₃): 9.63 (s, β -meso H), 9.38 (s, 8.58 (s, ô-meso H), 7.92 (dd, J 19.5, 12.8 Hz, 2a-H), 7.2 Hz, 4-b Me), 705 19.5 Hz, 2b-H), 6.18 (dd, J 12.8 Hz, 2b'-H), UV/Vis: (λ max/nm, ε): 0.08 and -0.17 (each br s, NH). 8.0 Hz, 8-Me), 1.65 (t, J 221-223°C.

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Purpurin-18-15 $^{
m l}$ -hexylisoimide methyl ester (7)

5.26 3.58 2.58-2.00 7b'-H; m, 7a-H; m, 7a'-H; m, hexyl isoimide-b,c-CH₂CH₂; 1.78 hexyl Ĕ, f-CH₃) (s, α-meso H), 8.65 (s, ô-meso H), 7.92 (dd, J 19.4, 12.2 Hz, 1.61 6.31 (dd, J 19.4 Hz, 2b-H), 6.18 (dd, J 12.2 Hz, 2b'-H), isoimide-CH₂), 3.83 (s, 5-Me), 3.76 (q, J 7.5 Hz, 4a-CH₂), Ĥ (E) hexylamide-d, e-CH2CH2), 0.96 (t. J 7.8 Hz, hexyl isoimide + H) Mp. 138-139°C. 1 H NMR $(\delta$ ppm, CDCl $_3$): 9.73 (s, β -meso (s, OMe), 3.46 (s, 1-Me), 3.28 (3-Me), 2.65 (m, 7b-H), (d, J 8.0 Hz, 8-Me), 1.72 (t, J 7.2 Hz, 4-b Me), 4.10 Ξ 8.5 Hz, 7-H), 4.57 (q, J 8.0 Hz, 8-H), -0.61 and -0.88 (each br s, NH). m/z (LRMS): 661 , ס

Purpurin-18- 13^{1} -hexyl isoimide methyl ester (6)

H₂), 3.56 .51-2.00 (s, α-meso H), 8.75 (s, δ-meso H), 7.94 (dd, J 19.2, 12.5 Hz, 2a-H), 6.33 (dd, J 19.2 Hz, 2b-H), 6.15 (dd, J 12.5 Hz, 2b'-H), 5.24 (d, J 8.5 Hz, 7-H), 4.52 (q, J 8.0 Hz, 8-H), 4.06 (t, hexyl S, NH) (m, 7b'-H; m, 7a-H; m, 7a'-H; m, hexylisoimide-b,c-CH₂CH₂; 7.2 Hz, 4-b Me), 1.58 (m, hexyl isoimide-d, e- CH_2CH_2), 0.98 dicyclohexylisourea-CH₂), 1.76 (d, J 8.0 Hz, 8-Me), 1.68 isoimide-a-CH₂), 3.81 (s, 5-Me), 3.74 (q, J 7.5 Hz, 4a-CH 7.8 Hz, hexylisoimide-f- CH_3), -0.66 and -0.84 (each br 1 H NMR ($^{\delta}$ ppm, CDCl $_3$): 9.74 (s, β -meso (s, OMe), 3.42 (s, 1-Me), 3.24 (3-Me), 2.65 (m, 7b-H), m/z (LRMS): 661 (M+H). Mp. 142-143°C.

Ester Dimethyl Purpurin-18-N-hexylimide-7-N-aspartylamide

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[Fisher's Nomenclature]

2b-H), 6.18 (dd, J 1 H NMR ($^{\delta}$ ppm, CDCl $_{3}$): 9.60 (s, eta -meso H), 9.34 (s, lpha -meso H), 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), 1.45 (m, N-hexylimideaspartate-CH₂), 2.64 (m, 7b-H), 2.51 (m, 7b'-H), 2.46 (m, 7a-H), 8.58 (s, ô-meso H), 7.90 (dd, J 19.8, 12.6 Hz, 2a-H), 6.98 (d, 2.06 (m, 7a'-H), 1.99 (m, N-hexylimide-b,c-CH₂CH₂), 1.75 (d, 4.38 -0.38 (3-Me), 5-Me), 3.69 (s, OMe), 3.64 (q, d, e-CH₂CH₂), 0.96 (t, J 7.8 Hz, N-hexylimide-f-CH₃), 7-H), (11,000); 549 (21,000); 510 (9,200); 483 (7,800); UV/Vis: (A max/nm, E): 705 4a-CH₂), 3.61 (s, OMe), 3.36 (s, 1-Me), 3.16 8.0 Hz, 9.6, aspartate-NHCO), 6.32 (dd, J 19.8 Hz, s, NH). m/z (LRMS): 791 (M+H) HZ, 8.5 **,** p) 5.34 (d, J 4.44 3.82 (s, hexylisoimide-a- CH_2), 2b'-H), Mp. 218-219°C. aspartate-CH), 0.11 (each br

Purpurin-18-N-hexylimide-7-N-Aspartylamide-di-tert-butyl

417 (110,000) (42,800); aspartate-CH), 8.58 (s, ô-meso H), 7.86 (dd, J 19.4, 12.5 Hz, 2a-H), 1 H NMR (δ ppm, CDCl $_3$): 9.63 (s, eta-meso H), 9.37 (s, 12.5 Hz, 2b'-H), 5.33 (d, J 8.5 Hz, 7-H), 4.66 (q, J 9.6 Hz, aspartate-NHCO), 6.32 (dd, J 19.4 Hz, 2b-H), 1-Me), (11,000); 549 (20,000); 510 (9,000); 483 (7,500); 705 's) UV/Vis: (\ max/nm, \(\epsilon\): 4a-CH₂), 3.36 4.41 (t, N-hexylimide-CH₂), 3.99 (m, 3.68 (q, J 7.5 Hz, 190-192°C. Mp.

2.46 1.37 (s, aspartate-^t Bu), 1.34 (s, aspartate-^t Bu), 1.14 (m, N-hexylimide-(m, 7a-H), 2.06 (m, 7a'H), 1.92 (m, N-hexylimide-b,c-CH₂CH₂), 38 and 7b'-H), regui d, e-CH₂CH₂), 0.95 (t, J 7.8 Hz, N-hexylimide-f-CH₃), -0. 1.68 (d, J 8.0 Hz, 8-Me), 1.66 (t, J 7.5 Hz, 4-b Me), 2.76 (m, aspartate-CH₂), 2.65 (m, 7b-H), 2.51 (m, (HRMS): 2/m C₅₁H₆₇N₆O₇: 875.5071. Found 875.5016. Mass: 0.11 (each br s, NH).

amide-di Purpurin-18-N-glycylimide-tert-Butyl Ester-7-aspartyla tert-butyl Ester (11): [Fisher's Nomenclature]

aspartate- t Bu), 0.10 and -0.04 (each br s, NH). m/z (LRMS) 905.4 aspartate-CH₂), 2.66 (m, 7b-H), 2.54 (m, 7b'-H), 2.46 (m, 7a-H), 4.68 (q, J 7.5 Hz, 8-H), 4.38 (m, aspartate-CH), 3.38 (s, 5-Me), 2.79 (m, meso H), 12.5 Hz, 2b'-H), 5.27 (d, J 8.5 Hz, 7-H), 5.18 (q, glycine-CH₂), 1.34 (s, (10,000); 549 (19,000); 510 (8,700); 483 (7,300); 417 (105,000) 65 (d, , dd, 2.01 (m, 7a'-H), 1.73 (d, J 7.5 Hz, 8-Me), 1.67 (t, J 7.5 Hz, (41,300); 1 H NMR (δ ppm, CDCl $_3$): 9.61 (s, eta-meso H), 9.35 (s, lpha-8.56 (s, 0-meso H), 7.88 (dd, J 19.5, 12.6 Hz, 2a-H), 6. 9.5 Hz, aspartate-NHCO), 6.26 (dd, J 19.5 Hz, 2b-H), 6.1 3.64 (q, J 7.5 Hz, 4a-CH₂), 3.35 (s, 1-Me), 3.16 (3-Me), Me), 1.58 (s, glycine-t Bu), 1.38 (s, aspartate-t Bu), UV/Vis: (λ max/nm, ε): 705 138-139°C.

408 (48,000); 363 (102,000). NMR (CDCl₃, à ppm): 9.21 (s, 1H, 5- H), Bacteriopurpurin-a 17-propyl Ester (12a): [Fisher's Nomenclature] UV/Vis (CH₂Cl₂, Amax, nm): 813 (56,000); 543 (32,000); 1 H, 20-H), 5.14 (d, 1H, J=8.0 (s, 1H, 10-H), 8.62 (s,

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2.14 (m, H, 17a-H), 1.98 (m, H, 17a'-H), 1.81, 1.73 (each CO₂CH₂), 3.66 (s, 3H, 12-Me), 3.55 (s, 3H, 2-Me), 3.17 (s, 3H, 2H, 3-H, 18-H), 4.08 (m, 1H, 8-H), 3.94 (t, 3-b Me), 8a-CH₂ 8.2, $CH_2CH_2CH_3$), -0.30 and -0.67 (each br + 7.8, Me), 2.73 (m, H, 17b-H), 2.41 (m, 5H, $\mathrm{CH_2CH_2CH_3}$ 3H, J = 8.0, 18-Me, 7-Me), 1.11 (t, 3H, J =(t, 3H, J

(17): Bacteriopurpurin-a-15¹-N-hexylisoimide Nomenclature):

8a-CH2 + 3H, 4H, hexylamide-f-CH₃), 0.88 (t, 3H, J = 8.2, CH₂CH₂CH₃), -0.68 and E) 5-H), 8.77 (s, 795 (67,000); 537 (24,500); 410 (50,400); hexylamide-d,e-CH₂, 1.11 (t, 3H, J = 7.8, 3-b Me), 0.97 8.0, 18-Me, 7-Me), 1.60 3H, 3-Me), 2.62 (m, H, 17b-H), 2.44 (m, 5H, CH₂CH₃ + 7b'-H), 2.14 (m, 6H, 17a-H + hexylisoimide-b,c-CH₂ + 10-H), 8.68 (s, 1H, 20-H), 5.35 (m, 2H, NHCO + 17-H), 4.11 (m, 3H, 8-H + hexylamide-a-CH₂), 3H, 2-Me), LRMS: 708 (M+H) (89,600). NMR (CDCl₃, b ppm): 9.21 (s, 1H, CO₂CH₂), 3.69 (s, 3H, 12-Me), 3.58 (s, s, 2H, 21, 23-NH). Mass: 11 1.93, 1.84 (each d, 3H, J 2Н, 3-Н, 18-Н), **Л**тах: 1.03 (each br W/vis:

(18) isoimide Bacteriopurpurin-a-13¹-N-hexyl Nomenclature

(33,200); 409 (59,400); 9.38 (s, 1H, 5-H), 5.46 (m, 1H, NHCO), UV/Vis (\lambda max/nm, \epsilon): 804 (82,800); 539 ï 1H, 10-H), 8.73 (s, 1H, 20-H), NMR (CDCl₃, δ ppm): 360 (94,000).

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1H, J = 8.0, 17-H), 4.34 (m, 2H, 3-H, 18-H), 4.17 (m, 1H, 8-H), 3.91 (m, 2H, hexylisoimide-a-CH₂), 4.06 (t, 2H, CO₂CH₂), 3.68 (s, 3H, 12-Me), 3.59 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2.73 (m, H, 17b-H), 2.42 (m, 5H, CH₂CH₂CH₃ + 8a-CH₂ + 7b'-H), 2.14 (m, H, 17a-H), 2.08 (m, 5H, hexylamide-b,c-CH₂+17a'-H), 2.01, 1.93 (each d, 3H, J=8.0, 18-Me, 7-Me), 1.57 (m, 4H, hexylisoimide-d,e-CH₂), 1.12 (t, 3H, J=7.8, 3-b Me), 0.96 (t, 3H, hexylamide-f-CH₃), 0.87 (t, 3H, J=8.2, CH₂CH₂CH₃), -0.86 and -1.13 (each br s, 2H, 21, 23-NH). Mass: LRMS:708 (M+1)

Biological Studies:

Determination of drug uptake:

ssue can pressure Xenon arc lamp and passes through a grating monochromator to a 90 a fixed The noninvasive number of time points after the i.v. injection of an experimental scatters light absorbing compound (e.g., a potential photosensitizer). current H2) we have ected by ble at fiber. (e.g., an experimental mouse tumor) and the light is coll contact with th at character of this measurement makes data collection possi The photo compound in living ti be recorded using an instrument and technique which high tuned tissue quartz ø The experiment measures the light distance (3 to 5 mm) from the first fiber. The light originates in amplified by The light signal is detected by a photodiode. second fiber placed in contact with the Hz chopper and then into a 400 μm diameter distal end of this fiber is placed in The absorption spectrum of a voltage, through the tissue. ø converted into developed.

amplifier and synchronously detected. The chopping at 90 Hz and synchronous detection makes examining spectra under normal room lighting possible.

absorption spectrum. The drug was then administered by tail vein contains a component caused by the presence of the drug in the anesthetized The optical tumor. This in vivo drug absorption spectrum is best displayed light signal which characterizes the instrument. As a safeguard against day to day or hour to hour drift in the total light output of the lamp, both spectra (pre- and post-injection) are normalized by dividing the signal strength at a wavelength where double beam absorption spectrophotometer. The pre-injection the by taking the ratio of the post-injection spectrum to the pre-(typically a cuvette and solvents) and the post-injection data spectra power as a function of wavelength was recorded before the the sample beam containing everything in the reference beam certainly not influenced by the wavelength dependence of injection spectrum. This ratio offers the same advantages The monochromator is set the reference beam second experimental drug's The ratio of these two first using either Pentobarbital or Ketamine Xylazine i.p. The these experiments, the mice were injection and the light signal recorded. can be thought of as expected longest wavelength of the the drug absorption is negligible. injection of the sensitizer. experimental drug. mouse data

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of Efficacy Vivo 18 using SMT-F Tumor Model Photosensitizing Efficacy: Bacteriochlorins 17 and

a mouse/tumor model reduction female DBA/2 Ha-DD mammary vivo via o growing spontaneous mouse A model system consisted of observing the size HA-DD mice. The tumor line was maintained in to male mice are readily available and were obtained locally. serial transplantation in the same mouse strain. The new photosensitizer was screened in subline, transplanted subcutaneously tumor, a fast of the tumor

Three tumor to recipient mouse. This technique provides for relatively tumor (1a donor the right and group. injected group mouse was experiments. mately. rown chosen from the auxiliary region of the animal within each experimental cube) were transplanted with a 18 gauge trocar from When tumor reached 4-5 mm in diameter, the animals were described above. Prior to irradiation, the fur overg surrounding the tumor was removed with electric clippers (approxi weeks) and weight (approximately 20 g), small pieces of uniform tumor size and allows location of the tumor in the chosen for drug, the When mice were both the appropriate animals with single tumors were potential photosensitizer injecting placed in a custom-made aluminum holder. after hours or twenty four with the

Irradiation Conditions:

guartz total to the Ø Standard light dose was 75 mW/cm² for 30 min for incident dose of 135 J/cm² from a tunable dye laser tune Spectra Physics 2040, absorption peak. red maximum

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Laser output was measured with dye the ţ a microlens was interfaced Further studies deliver a uniform field of light. fitted with meter. fiber

doses

light

at various

treatment conditions are currently in progress.

Experimental Procedure:

ಇರ reguire tap water tumor Following light exposure, the mice were kept in groups days tumor overlying surrounding skin was monitored daily for 80 both non-responsive and of food gross appearance pelleted photoillumination unless growth of early sacrifice of those animals. with and supplied size Tumor and cage

The photosensitizer was dissolved in known quantity of Tween 80 (Aldrich) surfactant and diluted by a factor of 10 with saline the the Absorption spectra were obtained 10-20%. of at basis o T photosensitizer final Tween 80 concentration the syringe solution was determined on using a Spectronic Genesis5 spectrophotometer. solution was then filtered through the oţ longest wavelength absorption. value coefficient solution to produce a concentration of the extinction

the Physics HPLC, connected with C8 reverse phase column, eluted with Spectra methanol/water by adjusting the pH to 7.0 using phosphate buffer of analytical HPLC using the drug into mice, compounds was ascertained by Before injecting the

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Alkylimide derivatives of bacteriochlorins:

N-hexylimide amines derivatives were followed except that various other alkyl o£ preparation t 0 were substituted for hexylamine. similar Procedures

antitumor The resulting compounds were tested for in vivo

tumors) were exposed to 75 mW/cm² for 30 minutes to deliver light The tumors were non-palpable and five mice were th SMT-F maximum red 4-6 mm diameter tumors (DBA/2 mice transplanted wi ď (135 J/cm^2) from a tunable dye laser tuned to absorption peak.

a large group The results indicate suppressive effect for isomers where the extended alkyl results are in Table 4. closest to the amino acid group.

and Part II - In vivo efficacy of bacteriochlorins 17 using Radiation Induced Fibrosarcoma (RIF) Tumor Model.

the and Each measurement was automatically recorded, where the tumor volume, V, was calculated er group Cal Mark evaluated for in vivo PDT efficacy using another model (RIF tumor moded) eated at least every other day thereafter, tumors were measured for PDT, routinely used in our laboratory. In brief, six mice p were tr orthogonal diameters with an electronic caliper (ultrachosen variable doses of light and drug. Beginning 24h after mixture of bacteriochlorins 17 and 18 was also animals mm) were experiment. As shown in Table 3, the III; Fred V. Fowler Co., Boston MA). appropriate tumor size (4-5

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The time for growth response was recorded on the basis of the number of animals which is the longest axis of the the tumor to 400 mm³ was then estimated by interpolation of were found to be tumor free. Appropriate controls were carried out with tumor-bearing mice which received no treatment reached. Was tumor and w is the axis perpendicular to 1. the times just before and after 400 mm^3 or received light or photosensitizer only. using the formula $v=(1w^2)/2$, where 1

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Table 1. Formation of purpurin-18 with cyclic anhydride and imide rings

ditions	
reaction cond	
various rea	
21 <	

Method No.	Conditions		Yield (%)	ж)
		imide 4	anhydride 1	starting materials (5 &6)
	THE, refluxing for 4h.	5	70	25
2.	Imidazole, 140°C, 1h	Decomp	Decomposition products	
3.	K-10 Clay, CH ₂ Cl ₂ , 24h	10-12	80-85	0
4.	CH ₂ Cl ₂ , 10 days	15-20	20-25	95
5. DCC with:			•	•
CO -1	K-10 Clay, RT		> C	200
	c. DBU, toluene, reflux 2h	9		40
5	RT, 10 min	85	0	0
6. TCD with:		,		
41 T	a. DBU, RT b. DBH rolune reflux 2h	o <u>c</u>	0 0	00 .
.	DBU, THF 60°C	30.	0	09
ਰ	d. KOH/MeOH RT, 10 min	01	0	06

DCC: Dicyclohexylcarbodiimide; DBU, 1,8-Diazabicyclo(5.4.0)undec-7-ene; RT, Room temperature; TCD, 1,1'-Thiocarbonyldiimidazole.

Table 2. Yields of purpurin-imides

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Showing response of Radiation Induced Table 3.

mice.	onse	14	808	408	808	*09	
of C3H	Tumor Response days	7	100\$	\$ 09	100\$	808	
ne skin o	Tume	1-2	100\$	808	100%	808	
(RIF) injected under the skin of C3H mice.	Timer after Injection hrs		24	24	24	24	
ожа	Light Dose Rate mW/cm ²		75	30	75	30	
Fibrosarc	Drug Dose mmol/kg		0.20	0.20	0.25	0.25	

TABLE 4

Antitumor Activity of Bacteriochlorins Comparative in vivo

Compound	Dose (µmol/kg)	in vivo absorption	Compound Dose in vivo Time(h) Tumor response (d) ^{†,} (µmol/kg) absorption betw. injection	Tumories	Tumor response (d)*.*
6 8 8 8 8 1 1		(А тах)	and light treatment	1-2	30 +
Mixture of 17 and 18	0.47	804	24	REGROWTH ON DAY 15)N DAY 15
20	0.47	804	24	NO RESPONSE	ONSE
22	0.47	804	24	REGROWTH ON DAY 4	N DAY 4
Mixture of 0.47 23 and 24	0.47	804	24	100 40	20

† 4-5 mm diameter tumors were exposed to 75 MW/cm² for 30 min to deliver 135 J/cm² light from a tunable dye laser tuned to the maximum red absorption peak.
d= days.

Non-palpable tumors.

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WHAT IS CLAIMED IS:

 A method for the manufacture of an imide derivative of purpurin comprising:

R₁₁ positions may contain at least one group selected from tituted having said macrocycle containing a and b rings which may be saturated or unsaturated at \mathbb{R}_4 to \mathbb{R}_{11} positions of the rings and which \mathbb{R}_4 aryloxy vative; thereto, arboxy with carbodiimide sqns deri reacting hexylamine with a chlorin or bacteriochlorin substituted fused substituent selected from carboxyl, hydroxy, phosphoro, purpurin group consisting of hydrogen, hydroxy, formal, and unsubstituted alkyl, alkoxy, alkenyl, aryl anhydride ring and reacting the purpurin derivative with wherein carbon containing groups may be amino and ether, to obtain a obtain the imide derivative of purpurin. six membered a macrocycle with a halo, sulfo,

2. The method of Claim 1, wherein the carbodiimide is dicycloberylcarbodiimide.

3. The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a purpurin imide of the formula:

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Asp-di of consisting and selected from the group consisting of OMe group the selected from Gly 13 and tert-butyl ester and R_l İs where R methyl hexyl

- 4. The method of Claim 1, wherein the imide derivative is further reacted with an alkali metal hydroxide to obtain a cyclic imide.
- 5. An imide of purpurin manufactured in accordance with the method of Claim 1.
- 6. An imide of purpurin manufactured in accordance with the method of Claim 2.
- 7. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 3.
- 8. A reaction product comprising an imide of purpurin manufactured in accordance with the method of Claim 4.
- A compound of the formula:

σ

a polyamine alkyl or aryl, or a carbonyl containing group, provided , where may be er with an amino taken together with $\mathtt{R_7}$ to form =0; $\mathtt{R_8}$ may be taken togeth that; R_4 may be taken together with R_5 to form =0; R_6 R_9 to form =0; R_{10} may be taken together with R_{11} to form alkyl; = 0 or = NR_{14} ; R_1 is an amino acid group, group; R_4 through R_{11} are -H, -OH, alkyl, alkylene, polyamine group, group, a polyether group or ${\tt OR}_{13}$ where ${\tt R}_{13}$ alkyl, substituted alkyl, a R₁₆ is H,

The compound of Claim 9 wherein R_{11} and R_{12} are -CH $_3$. 10.

together form a chemical bond; and \mathtt{R}_{12} is hydrogen or

 R_4 and R_7 may together form a chemical bond and R_8 and

alkyl; provided that if one z is = 0, the other z is = NR_1

- The compound of Claim 9 wherein R_2 is an allyl group. 11.
- The compound of Claim 9 wherein $R_{
 m j}$ is an allyl group. 12.
- The compound of Claim 9 having the formula: 13.

where R is normal alkyl of 2 through 12 carbon

The compound of Claim 9 having the formula:

alkyl of 2 through 12 where R is normal

R_{ll} may

- is o≕coR₁₅ of Claim 10 wherein R_2 The compound 15.
- The compound of Claim 10 wherein R_3 is 0=COR $_{15}$ 16.
- The compound of Claim 15 wherein R₁ 17.
- The compound of Claim 16 wherein \mathtt{R}_2 18.
- method for the manufacture of an isoimide derivative purpurin comprising reacting a purpurin of 19.

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=0; R₁₀ bstituted through R₁₁ or polyether may taken together with form a chemical bond and R_{B} and R_{11} may together form a ided that to open may alkyl and R₄ and R₇ 4 form if one z is =0, the other z is =NR $_{14}$, with 1-hexylamine carbonyl containing group, provided that; R carbodi bond; and R_{12} is hydrogen or lower alkyl; prov Ø alkyl, a polyamine group, or an amino acid group; $R_{f 4}$ alkyl, or a polyamine group, t C are -H, -OH, alkyl, alkylene, -OR $_{
m 16}$, where R $_{
m 16}$ to form =0; R₈ may be taken together with R₉ the anhydride ring followed by reaction with ; 0= taken together with R_S to form =0; R₆ may be $o_{R_{13}}$ where R_{13} is alkyl; R_{14} is obtain the isoimide derivative of purpurin. taken together with R₁₁ to form wherein R₁ is an amino acid group, ៧ group or aryl, or together chemical

20. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 9 with alkali metal hydroxide.

21. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 10 with an alkali metal hydroxide.

22. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 15 with an alkali metal hydroxide.

23. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 16 with an alkali metal hydroxide.

24. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 17 with an alkali metal hydroxide.

25. A method for the manufacture of purpurin imide comprising reacting the compound of Claim 18 with an alkali metal hydroxide.

26. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide.

27. A method for the manufacture of purpurin imide comprising reacting the purpurin derivative from Claim 19 with alkali metal hydroxide.

28. A method for the manufacture of an imide derivative of purpurin comprising:

positions may contain at least one group selected from the group reacting hexylamine with a chlorin or bacteriochlorin having saturated substituent carboxy, halo, sulfo, thereto substituted aryloxy, membered anhydrid ring fused þe unsaturated at R_4 to R_{11} positions and which ø amino and ether, to obtain a purpurin derivative; and said macrocycle containing a and b rings which may with aryl and formal, substituted selected from carbonyl, hydroxy, phosphoro, alkenyl, of hydrogen, hydroxy, containing groups may be alkyl, alkoxy, macrocycle with a six unsubstituted consisting carbon

reacting the obtained purpurin derivative with a carbodiimide to obtain the imide derivative of purpurin.

AMENDED CLAIMS

[received by the International Bureau on 9 September 1997 (09.09.97); original claims 1, 2, 9, 11, 17 and 19 amended; original claims 12, 15, 16, 18, 22, 23 and 25 cancelled; remaining claims unchanged (6 pages)]

of derivative imide аn of manufacture the purpurin comprising: method

lorin having Saturated substituted thereto, aryloxy derivative; carboxy, substituted with and which carbodiimide selected membered anhydride ring fused and substituent selected from carboxyl, hydroxy, phosphoro, pe reacting hexylamine with a chlorin or bacterioch] purpurin macrocycle containing a and b rings which may the rings formyl, aryl group alkenyl, and reacting the purpurin derivative with and R₁₁ positions may contain at least one hydroxy, containing groups may be amino and ether, to obtain a or unsaturated at \mathbb{R}_4 to \mathbb{R}_{11} positions of obtain the imide derivative of purpurin. alkyl, alkoxy, group consisting of hydrogen, Six ø and unsubstituted with carbon macrocycle halo, sulfo, wherein

- . T iimide carbod the wherein 1, Claim dicyclohexylcarbodiimide. of The method ٠ د
- obtain derivative to metal hydroxide of Claim 1, wherein the imide reacted with an alkali purpurin imide of the formula: method . ო

AMENDED SHEET (ARTICLE 19)

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where R is selected from the group consisting of OMe and Asp-digroup consisting selected from the Gly is and tert-butyl R₁ and ester methyl hexyl

- wherein the imide derivative metal hydroxide to obtain further reacted with an alkali Claim 1, The method of imide 4.
- with accordance 디 purpurin manufactured of method of Claim An imide
- with accordance in manufactured purpurin of method of Claim 2 imide •
- purpurin of imide of an manufactured in accordance with the method comprising product reaction 4
- purpurin đ O imide Claim in accordance with the method of an comprising product reaction manufactured
- compound of the formula: 6

AMENDED SHEET (ARTICLE 19)

polyamine alkyl is an amino acid group, OR_{13} where R_{13} is or = NR_{14} ; R_{1} group a polyether oH 0 fl j.s where z

amino acid with and ether with to form =0; R_6 may be taken together with R_{γ} to form =0; R_8 be taken alkenyl, group, chemical bond and R_{11} may together form a chemical bond; together with R_{11} to form =0; and R_4 and R_7 may together form amino substituted containing one alkoxy, ether substituents, provided that; $\mathtt{R_4}$ may be taken tog taken together with R_{9} to form =0; R_{10} may sulfo, an alkyl; provided that if or group; R_4 through R_{11} are -H, -OH, alkyl, substituted alkyl, a polyamine group, carbonyl, hydroxy, phosphoro, carboxy, halo, alkylene, aryl, or aryloxy, or a carbonyl wherein carbon containing groups may be and R₁₂ is hydrogen or lower 0, the other z is = NR_{14} . alkyl,

- The compound of Claim 9 wherein R_{11} and R_{12} are -Cl 10.
- The compound of Claim 9 wherein R_9 is -COCH₃. 11.
- The compound of Claim 9 having the formula: ы ы

where R is normal alkyl of 2 through 12 carbon atoms.

AMENDED SHEET (ARTICLE 19)

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The compound of Claim 9 having the formula

2 through 12 carbon atoms. where R is normal alkyl of

- is H₃CO₂C-The compound of Claim 9 wherein R_1 17.
- isoimide derivati purpurin comprising reacting a purpurin of the formula aл of the manufacture method for 19.

AMENDED SHEET (ARTICLE 19)

alkyl; R_{14} is polyamine in amino acid ø group, substituted alkyl, a polyamine group, or a 7.S is =0 or NR_{14} ; R_{1} is an amino acid where OR_{13} or polyether group wherein z Ó Ø alkyl,

and containing group, alkoxy, alkenyl amino substituted sulfo R_4 through R_{11} are -H, -OH, alkyl, carbonyl, hydroxy, phosphoro, carboxy, halo, alkylene, aryl, or aryloxy, or a carbonyl groups may be carbon containing wherein dronb;

together with form =0; R_B with R_7 to taken ether substituents provided that; \mathtt{R}_4 may be form form =0; R₆ may be taken together Ç

=0; R₁₀ may be taken together with \mathtt{R}_{11} to form =0; and \mathtt{R}_4 and \mathtt{R}_7 may together form a chemical bond; chemical bond and $R_{
m B}$ and $R_{
m Il}$ may together form a taken together with Rg to

anhydride one z is =0 obtain open the t 0 and R_{12} is hydrogen or lower alkyl; provided that if carbodiimide z is =NR₁₄, with 1-hexylamine to Q followed by reaction with isoimide derivative of purpurin.

comprising reacting the compound of Claim 9 with alkali metal hydroxide. A method for the manufacture of purpurin imide 20.

comprising hydroxide A method for the manufacture of purpurin imide reacting the compound of Claim 10 with an alkali metal 21.

comprising hydroxide meta] imide purpurin reacting the compound of Claim 17 with an alkali method for the manufacture of

comprising with alkali metal the manufacture of purpurin imide reacting the purpurin derivative from Claim 19 method for hydroxide.

comprising reacting the purpurin derivative from Claim 19 with alkali 27. A method for the manufacture of purpurin imide hydroxide

AMENDED SHEET (ARTICLE 19)

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of derivative an imide of manufacture the purpurin comprising: for method

reacting hexylamine with a chlorin or bacteriochlorin having positions may contain at least one group selected from the group alkenyl, aryl and aryloxy, wherein selected from carbonyl, hydroxy, phosphoro, carboxy, halo, sulfo, substituent formal, substituted which R₄ six membered anhydrid ring fused said macrocycle containing a and b rings which may be carbon containing groups may be substituted with a amino and ether, to obtain a purpurin derivative; and and positions hydrogen, hydroxy, or unsaturated at R_4 to R_{11} unsubstituted alkyl, alkoxy, macrocycle with a ţ0 consisting

đ carbodiimide to obtain the imide derivative of purpurin purpurin derivative the obtained reacting

СН2СН3

CH3

(wu 969) 9 (mn 207)4 $(CH^{2})^{2}CH^{2}$ 3. R=COOH, $R^1=CONH(CH_2)_5CH_3(Minor)$ 2. $R = CONH(CH_2)_5CH_3$, $R^1 = COOH(Major)$ (mn 007) 1 hexyl amine

Light/air

CO₂CH₃

CO₂Phytyl

Bacteriochlorophyll—a,

FIG 2

Chlorin, 660 nm CO₂CH₃

K-10 CLAY

и́н(сн₂)₅сн₃ (Main product) CH² ΗŅ ΗŅ

FORMATION OF CYCLIC ANHYDRIDE

FIG 3

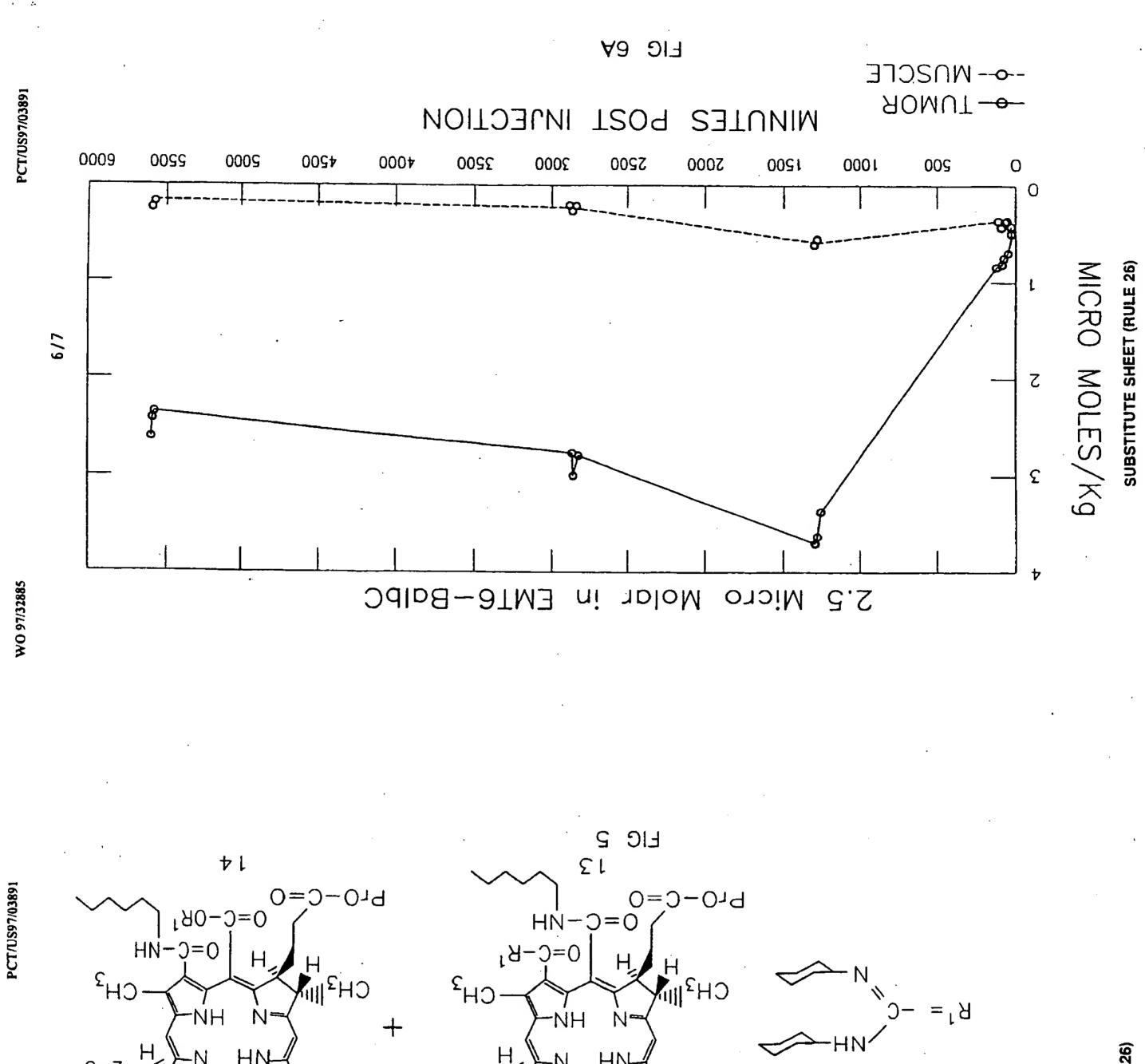
$$H^{2}CO^{5}C$$
 $H^{2}CO^{5}C$
 H^{2

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MeOH

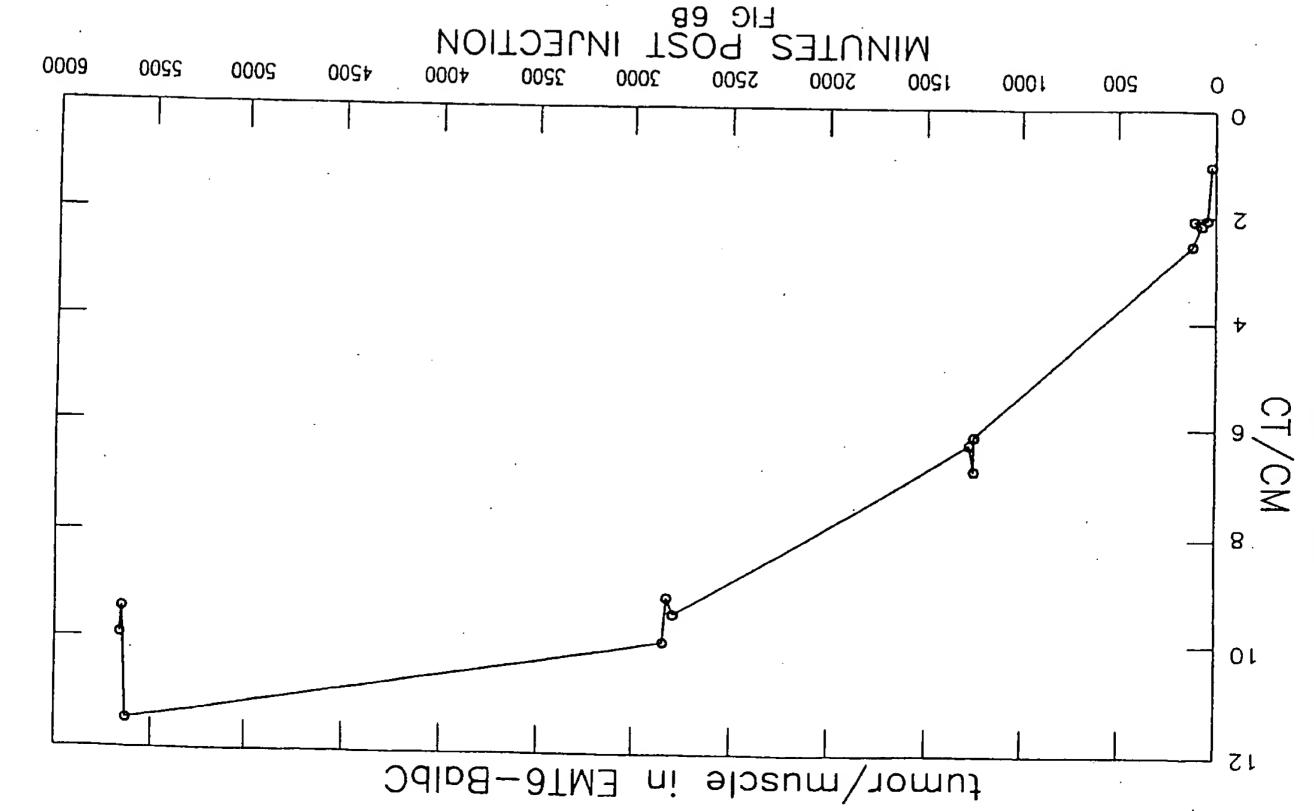
КОН

FORMATION OF CYCLIC IMIDE



H HM HM CH²-CH²-5/7 CH 15 01 Pr0-C=0 Pr0-C=0 $\dot{C}O_{2}H$ O = C - NH $co^{S}H$ O = C - NHCH³III HCH²III CH^{2} CH^{3} CH^2 ŃН ŃН ·ΝΗ H HN HN HM ÇH³− CH²-CH²-0 CH CH





1-4 and 20-28. Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Relevant to claim Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) International application No. 1-28 1-28 PCT/US97/03891 5,591,847 A (PANDEY et al) 07 JANUARY 1997 see FIESER AND FIESER, Reagents for Organic Synthesis, Vol LEE et al. Use of the Chlorophyll Derivate, Purpurin-18, for Syntheses of Sensitizers for Use in Photodynamic Therapy., Jorn. Chem. Soc, Perkin Trans I. October 1993, pages 2369-See patent family annex. Citation of document, with indication, where appropriate, of the relevant passages 2372, especially page 2369 and formulae on page 2370. US CL | 540/145,472,474
According to International Patent Classification (IPC) or to both national classification and IPC Minimum documentation searched (classification system followed by classification symbols) 1,1967, page 233. N.Y. John Wiley & Sons. INTERNATIONAL SEARCH REPORT Further documents are listed in the continuation of Box C. DOCUMENTS CONSIDERED TO BE RELEVANT CLASSIFICATION OF SUBJECT MATTER entire document. FIELDS SEARCHED 540/145,472,474 C07D 487/22 U.S. Category* ۵ × < × >

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Washington, D.C. 20231

Date of mailing of the international search report

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Date of the actual completion of the international search

02 JUNE 1997

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HJCOJC

1 (700 am)

HJCOOCH

CONVERSION OF BACTERIOCHLOROPHYLL.A INTO CHLORIN IN PRESENCE OF LIGHT AND AIR

2. $R_2 = CONH(CH_2)_5CH_3$. $R_3 = COOH$ (Major) 3. $R_3 = COOH$. $R_2 = CONH(CH_2)_5CH_3$ (Minor)

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Light/Air

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INTERMEDIATE

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ČO₂CH₃

CO2Phytyl

COCCH3

CO2Phytyl

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Chlarin, 690 nm

Bacteriochlorophyll-a , 780 nm

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HJCOJC

Medhikoh

MeOH/KOH

Ç HJ-

CH2CH3 Ę,

R = CONH(CH₂)₅CH₃

FIG. 2

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HJCOJC

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Clay

 $\text{CO}^{\text{S}}\text{CH}^{3}$ 3/7 €HD-(Main Product) CH^SCH³.

EIG 3

H³C

$$H_3C$$
 H_3C
 $$H_3C$$

$$H_3$$

$$CH_2)_2CH_3$$

$$CH_2)_2CH_3$$

$$CH_2)_2CH_3$$

$$CH_2)_2CH_3$$

$$CH_2)_2CH_3$$

$$DCC$$

$$H_3C$$

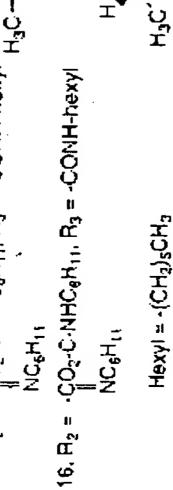
$$DCC$$

$$H_3C$$

$$C_2H_3$$

$$C_2H_3$$

OOF F



INTERMEDIATE



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ивилтез михт вивстон

DOOZ

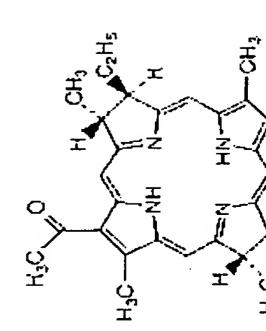
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2.5 Micro Molur in EMT6-BulbC

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DCC: 1,3-Dicyclohexylearbodiimide $\mathsf{R}_2 = \mathsf{R}_3 = \cdot (\mathsf{CH}_2)_{\mathsf{S}} \mathsf{CH}_{\mathsf{S}}$

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Pr_{O₂C}

MIN2CLE TUMOR

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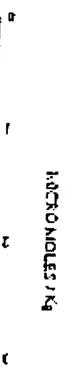


FIG. 5



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